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VISCERAL LESIONS IN INFECTIOUS POLYNEURITIS

(INFECTIOUS NEURONITIS, ACUTE POLYNEURITIS WITH FACIAL DIPLEGIA, GUILLAIN-BARRÉ SYNDROME, LANDRY'S PARALYSIS)*

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Infectious polyneuritis or neuronitis is the name usually applied to a disease which is characterized by widespread motor and sensory signs pointing to involvement of spinal and cranial nerves (most commonly the seventh) which usually appear after an interval of days or weeks following a mild respiratory infection. An important diagnostic feature is the marked increase in the protein of the cerebrospinal fluid without any change in the number of cells.† While the disease has been observed in persons of almost all ages, it has proved fatal, thus far, only in older adults,‡ and the survivors, as a rule, make excellent recoveries. Although there are some reports [1] which maintain that the nervous system exhibits no pathologic change in this disease, it appears to be fairly well established [2-4] that definite degeneration affecting the axis cylinders and myelin sheaths, with or without inflammatory reaction, is demonstrable in the peripheral nerves, and that in the

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† It is our belief, however, that certain patients suffering from this disease may have some pleocytosis.

‡ Since this paper was submitted for publication we have encountered a fatal case of this disease in a boy, 4½ years old, who died in November 1940 with a clinical diagnosis of poliomyelitis. Histological examination of the nervous system revealed no lesions of poliomyelitis, while changes characteristic of infectious polyneuritis were found in the nerve roots.

central nervous system the cell bodies may exhibit varying degrees of chromatolysis and eccentricity of the nuclei. With the exception of some cellular infiltration in the peripheral nerves and sensory ganglia, no inflammatory reaction has been observed in the nervous system. The etiology of this form of polyneuritis is unknown, but, despite the fact that an early report [2] of transmission to monkeys has not been confirmed, the statement is often made that the disease is caused by an unknown filtrable virus.

The present study was begun with the intention of obtaining some orienting data concerning the etiology of the disease. All the previous fruitless attempts at transmitting the disease to animals were made with nervous tissue. Since it has recently been shown [5] that a new member of the pleuropneumonia group of filtrable microörganisms can, while multiplying in certain visceral cells of mice, produce a toxin with a selective affinity for specific parts of the nervous system, it appeared worth while not only to culture the viscera in fatal cases of infectious polyneuritis but also to observe the effect of inoculation of the fresh tissues into various animals. It was in the course of such a study that histologic examination of the human viscera revealed the lesions to be described in the present communication.

Report of Cases

The clinical histories of the three patients whose viscera were examined will be recorded briefly to indicate that they all exhibited the characteristic manifestations of the disease (Table I).

Case 1

J. S., a white male, 40 years old, was well until February 4, 1940 when he developed a "head cold" (sneezing, nasal discharge, sore throat) and complained of "rheumatism" and pain in the lumbar region. Apparently the initial symptoms did not persist long, but on February 13, after a period of relative well-being, he developed headache, malaise and tingling in the hands and feet. The next day he was obliged to go to bed because of weakness of his legs. On February 15 he had no fever but his legs, arms and hands were very weak, and on February 16 he experienced cramplike pains in the thighs and hips and to a lesser degree in the shoulders and arms. On February 17 he still had no fever, but he was weaker, and mucus began to collect in his throat. He was admitted to the hospital on February 18, exhibiting complete flaccid paralysis of the legs and arms, bilateral facial paralysis, dysphagia, and difficulty in respiration. He was put into a respira-

TABLE I

Clinical Data on Three Cases of Infectious Polyneuritis with Visceral Lesions

	Age	Color and sex	Primary illness	Initial nervous symptoms	Spinal nerve involvement			Cranial nerve involvement						Cerebrospinal fluid		Throat culture	Died
					Tendon reflexes	Sensory signs and symptoms	Paralysis	Fifth nerve	Facial palsy	Palatal paralysis	Voice disturbance	Respiratory difficulty	Dysphagia	Cells	Total protein mg.%		
1 J.S.	40	W.M.	"Head cold" and "rheumatism," 2/4/40	Headache, tingling in hands and feet, 2/13/40	Arms, o Legs, o	Pain and tenderness; sensory examination not done	Arms, + Legs, +	Bilateral motor weakness	Diplegia	Partial	Hoarse, nasal	Yes	Yes	3 l.	125	No diphtheria bacilli	2/21/40
2 A.M.	46	W.F.	Upper respiratory infection and myalgia, 2/15/40	Nausea, 3/1/40; vomiting, 3/7/40; weakness and numbness of legs, 3/8/40	Arms, o Legs, o	Pain, tenderness, paresthesias; impaired touch, vibration and position	Arms, + Legs, +	Tenderness of jaws; bilateral motor weakness	Diplegia	Complete	Hoarse, nasal	Yes	Yes	o	80	No diphtheria bacilli	3/11/40
3 G.F.	56	W.F.	"Chest cold," 2/11/39 to 2/14/39	Pain, numbness, weakness both legs, 2/27/39	Arms, o Legs, o	Pain, numbness, tenderness; impaired vibration	Arms, + Legs, +	Hypalgesia, left; bilateral motor weakness	Diplegia	?	Nasal	Yes	Yes	o 4 l.	130 75	Not done	3/11/39

tor on February 19, but after increasing weakness and restlessness he died on February 21, 8 days after the first appearance of nervous symptoms.

His temperature varied from 100° to 101° F. on February 18, from 100° to 98.8° F. on February 19, from 98.8° to 100.5° F. on February 20, and rose to 102° F. just before death on February 21. The blood Kahn reaction was negative. Two throat cultures were negative for diphtheria bacilli. The cerebrospinal fluid obtained on two occasions was colorless, clear, and contained respectively 100 mg. and 125 mg. of protein per 100 cc., but there was no increase in cells. Only in the last 2 days of life was the pulse disproportionate to the temperature, varying from 104 to 148. The white blood count was 23,000 per cmm., with 97 per cent polymorphonuclear cells.

Case 2

A. M., a white female, 46 years old, engaged in housework, developed a mild upper respiratory infection with nonproductive cough and myalgia on February 15, 1940. She continued to work, however, and felt better in a few days. On March 1 she developed anorexia and nausea severe enough to prevent her from eating, and on March 7 she began to vomit. The vomiting was neither projectile nor related to eating, but was brought on by coughing. On March 8 she first noticed that her legs were weak and felt "dead." On admission to the hospital on March 9 she complained also of numbness and tingling in the arms, and exhibited bilateral facial palsy and flaccid paralysis of the legs and arms. Difficulty in swallowing and in respiration appeared on March 10, and she died in the respirator on the morning of March 11, 3 days after the first appearance of weakness in the extremities.

The temperature was 100.2° F. on admission (March 9), but below 99° F. thereafter. The pulse rate was 80 to 88 on March 9, and, in association with a normal temperature, 118 to 122 on March 10 and 11. The Wassermann reaction was negative on the cerebrospinal fluid. The blood count was normal, and a throat culture was negative for diphtheria bacilli. The cerebrospinal fluid was colorless and clear, and contained 65 mg. sugar, 703 mg. chlorides, and 80 mg. of protein per 100 cc., but no cells were present. The urine contained reducing substance and acetone on admission (the latter perhaps due to starvation), but no albumin. The blood sugar determined on two occasions was 123 and 126 mg. per 100 cc.

Case 3

G. F., a white male, 56 years old, a stationary engineer of a bakelite plant, developed a "chest cold" with a slightly productive cough on February 11, 1939. For the following few days he did not feel well but he continued to work. Between February 14 and February 25 he "never felt better in his life," but on February 27 he suddenly developed pain, weakness and numbness in both legs. By March 2 both the lower and upper extremities were affected, and on March 4 he was admitted to the hospital exhibiting, as predominant symptoms, weakness of all extremities and bilateral facial paralysis. His condition became progressively worse, and he developed paralysis of the eye muscles and of the jaw, and finally respiratory difficulty. He died on March 11, 12 days after the appearance of nervous symptoms.

The temperature was normal until the last day of life when it rose to

100° F. and reached 102° F. just before death. The pulse rate was 80 to 90 until March 8, 96 to 120 thereafter and 140 before death. The blood Wassermann reaction was negative and the blood count was normal. The cerebrospinal fluid was colorless and clear on two occasions, and contained respectively 130 and 75 mg. of protein per 100 cc., but there was no increase in cells. Examination of the urine revealed nothing abnormal.

Animal Inoculations and Cultures with Tissues Obtained from Case 1

Pieces of the liver, spleen, kidneys, adrenals, and of the peripheral portions of the lungs were obtained under aseptic precautions within 2 hours after death. They were streaked on 30 per cent ascitic fluid agar plates, which were examined periodically under the microscope for evidence of development of colonies of the pleuropneumonia group. These cultures were negative. While still fresh, the viscera were pooled, ground with sand, and suspended in physiologic salt solution. After light centrifugation, the following inoculations were made: 0.03 cc. intracerebrally and 1 cc. intra-abdominally into each of 8 *mice,* 18 days old; 0.2 cc. intracerebrally and 2 cc. intra-abdominally into each of 3 *guinea pigs* weighing 250 gm.; 0.5 cc. intracerebrally and 5 cc. intra-abdominally into each of 2 *rabbits;* 2 cc. intracerebrally, 10 cc. intra-abdominally, 0.2 cc. intracutaneously into two areas of the abdominal skin of a *rhesus monkey.* Pieces of the spinal cord and medulla, also obtained under aseptic precautions, were similarly prepared and inoculated in the same manner into the same kinds and numbers of animals. The mice and rabbits remained well during 8 weeks of observation, the monkeys during 11 weeks, and the guinea pigs over a period of 18 weeks. Passage of various tissues from some of the inoculated mice into new mice also gave negative results.*

Changes in the Nervous System Hitherto Undescribed

Examination of the peripheral nerves in all three cases revealed the changes which have already been recorded by many observers; *i.e.,* degeneration of the axis cylinders and myelin sheaths, proliferation of the cells of the sheath of Schwann, and intersti-

* The viscera and nervous tissue of the boy, 4½ years old, who died of this disease in November 1940 were similarly cultured on 30 per cent ascitic fluid agar with negative results. Rhesus monkeys and mice inoculated with the nervous tissue (medulla or olfactory bulbs) remained well.

tial infiltration with various inflammatory cells. Interstitial infiltration with mononuclear cells was also found in the intervertebral ganglia as well as in the gasserian ganglia (Fig. 7) which were studied in case 1. Certain other observations have been made, however, which have not been recorded hitherto. For example, reference is often made to the chromatolysis which can be seen in the nerve cells of the anterior horn of the spinal cord and in certain nuclei of the medulla, and which has been interpreted by some observers as merely postmortem change. Sections of tissue obtained from case 1 soon after death and stained with eosin and methylene blue after fixation in Zenker's fluid with 5 per cent acetic acid revealed a rather striking "zonal chromatolysis" (Figs. 1 and 2). The Nissl substance appeared well preserved in most of the cell with the exception of a distinctly circumscribed zone of varying size which at first suggested an acidophilic, cytoplasmic inclusion but on closer observation could be identified as an area devoid of Nissl substance. In Nissl preparations these zones appeared as vacuoles and were seen in sections from all three cases.

Examination of a number of sections from both olfactory bulbs of case 1 revealed a vessel in the glomerular layer of one of the bulbs with typical cuffing by mononuclear cells (Fig. 6). This finding was particularly puzzling since no such perivascular infiltration was seen in sections from any other part of the nervous system. Whether or not this observation has any special significance in infectious polyneuritis cannot be decided until more olfactory bulbs have been examined in this disease, but it is of interest to note that even in human poliomyelitis such infiltration has been recorded only twice in a study of serial or semiserial sections of forty-nine olfactory bulbs.[6]

Another finding, of which the significance for infectious polyneuritis can be determined only by future studies, was present in the abdominal sympathetic ganglia of the celiac plexus in case 1 (Zenker's-acetic fixation and eosin-methylene blue stain). A relatively small number of cells exhibited a peculiar degenerative lesion (Figs. 3–5). The striking change consisted not so much of a progressive vacuolization (vacuolization of occasional sympathetic ganglion cells has been noted[7] in various unrelated conditions), but of the presence of deep-staining, sharply out-

lined, acidophilic bodies (black bodies in the photomicrographs) distributed throughout the cytoplasm and especially within the vacuoles. The vacuolization appeared to begin at the periphery of the cell (Fig. 3) and to continue toward the nucleus until most of the cytoplasm had been affected (Fig. 4), leaving a structure which still had the outline of the original cell but showed only vacuoles and many red bodies (Fig. 5). One of us (A. B. S.) has observed similar, though somewhat smaller, acidophilic bodies in nerve cells undergoing necrosis as a result of infection in mice with the virus of equine encephalomyelitis. In addition to the cellular changes just described, there was an occasional rather small focus of infiltration with various types of mononuclear cells (Fig. 8). Such focal, interstitial, cellular infiltration has recently been reported [7] in the sympathetic ganglia of patients dying from a varied number of infectious diseases.

Visceral Lesions

Adrenals. The most marked changes in the adrenals were found in case 1. Grossly, the left adrenal showed marked degeneration, having the consistency of a fluid-filled bag, while the right adrenal was well preserved. Microscopically, lesions were present in both. In addition to the marked degeneration and hemorrhage at the cortico-medullary junction in the left adrenal, three distinct types of lesions were observed: (a) foci of degenerated cortical cells associated with an infiltration of various mononuclear cells (Fig. 9); (b) infiltration with mononuclear cells along the adrenal nerves (Fig. 10); (c) focal accumulations of lymphocytes and plasma cells occupying what appeared to be dilated lymphatics or the sites of degenerated cortical cells, or both (Fig. 12). It should be noted that these changes were found only because sections were obtained from various parts of the adrenals, and that some sections showed no lesions at all. Case 3, in which only one zone was sectioned, exhibited more interstitial infiltration with mononuclear cells than was seen in case 1, and in addition many foci of vacuolization of cortical cells (Fig. 11). One section in case 2 showed only focal vacuolization. Additional sections from the same block, however, revealed focal cellular infiltration at the cortico-medullary junction, and focal phlebitis of the type found in the heart and lungs of the same case.

Liver. The following types of lesions were observed on microscopic examination (Figs. 13–17): (a) focal cellular infiltration in Glisson's capsule, consisting predominantly of mononuclear elements (case 1, Fig. 13); (b) infiltration of interlobular connective tissue or portal canals with mononuclear and occasionally polymorphonuclear cells (cases 1, 2 and 3, Figs. 14 and 15); (c) focal necrosis involving a few liver cells and infiltration of the site with mononuclear and few polymorphonuclear cells (cases 2 and 3, Figs. 16 and 17); (d) focal fatty degeneration and infiltration (cases 1, 2 and 3, Fig. 16).

Here, it is necessary to point out again that these lesions can be missed unless sections are obtained from more than one region of the same organ, or multiple sections from the same block are examined. The least change was found in case 3, in which multiple sections were required to reveal the described lesions, while in case 1, of four regions examined, two showed no change beyond that mentioned in (d).

Heart. The most severe and extensive lesions (Figs. 18–22) in the heart were found in case 2, and consisted chiefly of: (a) diffuse interstitial infiltration with mononuclear and polymorphonuclear cells (Figs. 18 and 21); (b) areas, suggesting necrosis of isolated muscle fibers, infiltrated by phagocytic cells, *i.e.*, foci of "myophagia" (Figs. 18 and 19); (c) focal phlebitis affecting portions of a coronary vein (Fig. 21), in which the wall was edematous and infiltrated with mononuclear and polymorphonuclear cells (Fig. 22).

Case 3 showed also focal interstitial cellular infiltration (Fig. 20), but of much less extent than that found in case 2, being present in sections from one region of the heart but not in those from another. It is also noteworthy that with the exception of the cellular infiltration, the muscle fibers showed no obvious evidence of degeneration in cases 2 and 3. In case 1, however, one could not be certain that there was no degeneration.

Kidneys. The characteristic lesion (Fig. 23), present most extensively in case 2, consisted of an interstitial infiltration with mononuclear cells especially between the tubules. The adjacent vessels were, as a rule, greatly congested, but the tubules and glomeruli themselves were well preserved. These changes were minimal in case 1, in which only two small foci were found in

one of three sections. In case 3, in addition to the scattered intertubular infiltration, there was complete fibrosis and obliteration of an isolated glomerulus suggestive of early nephrosclerosis. The pathologic changes in these kidneys correspond exactly to those described by Councilman,[8] especially in certain patients with diphtheria or scarlet fever.

Lungs. There was an acute bronchitis and lobular pneumonia in all three cases, probably the result of aspiration of foreign material in the last days of life. Case 2, which exhibited focal phlebitis in the heart and adrenals, showed a similar change in one of the pulmonary veins.

Other Organs. The spleen in all cases showed marked engorgement of the pulp with red cells, phagocytosis of erythrocytic elements, diminution in size of the malpighian bodies, and slight infiltration of the reticulum with polymorphonuclear leukocytes. There were no other significant findings with the possible exception of a mild, focal, acute colitis in case 3.

TABLE II

Distribution of Visceral Lesions in Infectious Polyneuritis

Patient	Adrenals	Liver	Heart	Kidneys
J.S. (case 1)	+++ (D., C. In.)	++ (C. In., D.)	− ?	+ (C. In.)
A.M. (case 2)	+ (C. In., D., Phleb.)	+++ (C. In., D., N.)	+++ (C. In., N., Phleb.)	++ (C. In.)
G.F. (case 3)	++ (C. In., D.)	+ (C. In., D., N.)	+ (C. In.)	++ (C. In.)

D. = degeneration; C. In. = cellular infiltration; N. = focal necrosis; Phleb. = phlebitis

A summary of the lesions found in the adrenals, liver, heart and kidneys (Table II) shows that, with the possible exception of the heart, each of these organs was affected in all three cases.* A search was made for inclusion bodies but none was found.

DISCUSSION

Acute infectious polyneuritis has been regarded as predominantly a disease of the nervous system, which it manifestly is from a clinical point of view. This viewpoint, however, has prob-

*We have observed similar visceral lesions in two additional cases which came to necropsy since this paper was submitted for publication.

ably been responsible for the failure to study the viscera in any detail in the past. We have been able to find but two reports in which the condition of the viscera is mentioned. In his communication on the pathology of the cases studied under the pressure of war conditions in 1917–1918, Bashford [2] stated: "The only change in the liver was a slight and variable infiltration of round cells in the large and small portal tracts such as is found in many febrile (infective) diseases. The kidneys in all cases showed early, patchy, parenchymatous and glomerular nephritis." In a necropsy report by J. Ganim quoted in McIntyre's [9] paper on infective neuronitis, there is a description of liver lesions which includes all the types which we observed, and it is also noted that there were degeneration of the heart muscle and vacuolization of the cortical cells in the fascicular zone of the adrenals. Our own studies on the viscera in infectious polyneuritis indicate that the adrenals, the liver, the heart and the kidneys may all be affected and that the pathologic changes correspond in type to those observed in such diseases as diphtheria, scarlet fever and typhoid fever, in which a toxin or toxins elaborated by specific microörganisms are believed to be responsible for the visceral manifestations. Our own interpretation of the lesions in the nervous system of patients with infectious polyneuritis is that the primary attack of the causative agent is on the peripheral nerves, which exhibit the oldest lesions, and that the changes found in the central nervous system represent the usual reaction to injury of the axis cylinders. When the primary attack is on the cell body of the neuron, as in the case of poliomyelitis or related virus diseases, the pathology is altogether different and the visible change in the peripheral nerves is the later manifestation. While the nature of the visceral lesions does not in itself exclude the operation of some unknown virus, the visceral and nervous changes taken together are unlike those which would be expected from the effects of a pantropic virus.

It was stated previously that the present study was undertaken for the purpose of obtaining orienting data concerning the etiology of the disease. No agent has been cultivated from the affected tissues since Wilson [2] has retracted his work after criticism by Arkwright,[10] nor has the disease been transmitted to animals since Bashford's [2] early inconclusive attempts. It is our

belief that the working hypothesis that may perhaps be pursued most profitably for the present is that infectious polyneuritis is caused by a toxin or toxins elaborated by the microörganisms which are responsible for the infection of the respiratory tract which usually precedes the onset of the nervous symptoms. To this end every attempt should be made to ascertain the bacterial flora in the respiratory tract in patients with this disease, and whether or not toxins which might reproduce the syndrome of infective polyneuritis are elaborated by those bacteria. It should be mentioned here that Bradford [2] originally reported that diphtheria bacilli were never found in any case of his series, nor have any been found by other investigators, including ourselves, since then. The interval elapsing between the original infection and the appearance of nervous symptoms is similar not only to that observed in the case of certain bacterial toxins, such as diphtheria, but also to that seen in the case of nonbacterial toxins such as are encountered in "Jamaica Ginger" poisoning in which the interval between drinking and the onset of symptoms is 7 to 14 days.[11]

The realization that infectious polyneuritis is caused by an agent or agents which can attack the viscera as well as the peripheral nerves leads one to inquire about the frequency with which the visceral lesions may be reflected in abnormal clinical signs or laboratory findings. Bradford,[2] for example, mentioned tachycardia of 100 to 160 with normal temperature and the patients lying quietly in bed; he also noted the presence of small quantities of albumin in the urine in the absence of pyrexia or catheterization and with no evidence of nephritis. It is noteworthy that in our own cases the heart rate was normal or proportional to the temperature except in the last 2 days of life, when the disproportionate increase in the rate might have been caused by nervous as well as by cardiac disorders, and that the urine did not contain albumin. It is not improbable, however, that the severity with which various organs are attacked may vary in different patients, and reference may be made to the case reported by McIntyre [9] in which the cause of death appeared to be on a cardiac rather than a nervous basis. However, more clinical and laboratory evidence of visceral involvement may follow the knowledge of existing pathologic changes in the internal organs.

Summary

Pathologic changes are reported in the viscera of three typical cases of infectious polyneuritis. The lesions in the adrenals consisted in the main of focal degeneration and infiltration with mononuclear cells; those in the liver of focal cellular infiltration in the capsule and portal spaces, focal necrosis of liver cells with cellular infiltration, and of focal fatty degeneration; those in the kidneys of focal intertubular infiltration with mononuclear cells, and those in the heart of interstitial infiltration with mononuclear and polymorphonuclear cells and in one case of necrosis of isolated muscle fibers and focal phlebitis. Focal phlebitis was also encountered in the adrenals and lungs of the same case. "Zonal" chromatolysis is described in the nerve cells of the spinal cord and medulla and a degenerative change consisting of vacuolization and the appearance of many acidophilic, sharply outlined bodies in the cytoplasm of some of the nerve cells in the abdominal sympathetic ganglia. Perivascular cuffing with round cells of a vessel in the glomerular layer of one of the olfactory bulbs was observed in the one case in which they were studied.

Mice, guinea pigs, rabbits, and rhesus monkeys were inoculated with a pool of the lungs, liver, spleen, adrenals and kidneys, and also with the spinal cord and medulla from one of the cases, with negative results. Cultures of these tissues for microörganisms of the pleuropneumonia group were also negative. The belief is expressed that the existing data fit best the hypothesis that infectious polyneuritis is caused by a toxin or toxins with affinities for the peripheral nerves and the viscera and elaborated by the microörganisms responsible for the infection of the respiratory tract which usually precedes the onset of nervous symptoms.

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DESCRIPTION OF PLATES

PLATE 85

FIG. 1. Anterior horn cells of spinal cord (case 1) showing "zonal chromatolysis." × 160.

FIG. 2. Anterior horn cell of spinal cord (case 1) showing "zonal chromatolysis." × 670.

FIGS. 3, 4, and 5. Nerve cells in abdominal sympathetic ganglia (case 1) showing various stages of degeneration. Note the dark, sharply outlined bodies (acidophilic) in the vacuolated cytoplasm. The lower cell in Figure 5 is an unaffected nerve cell in the same field for comparison. × 1030.

All photomicrographs were made by Joseph B. Homan.

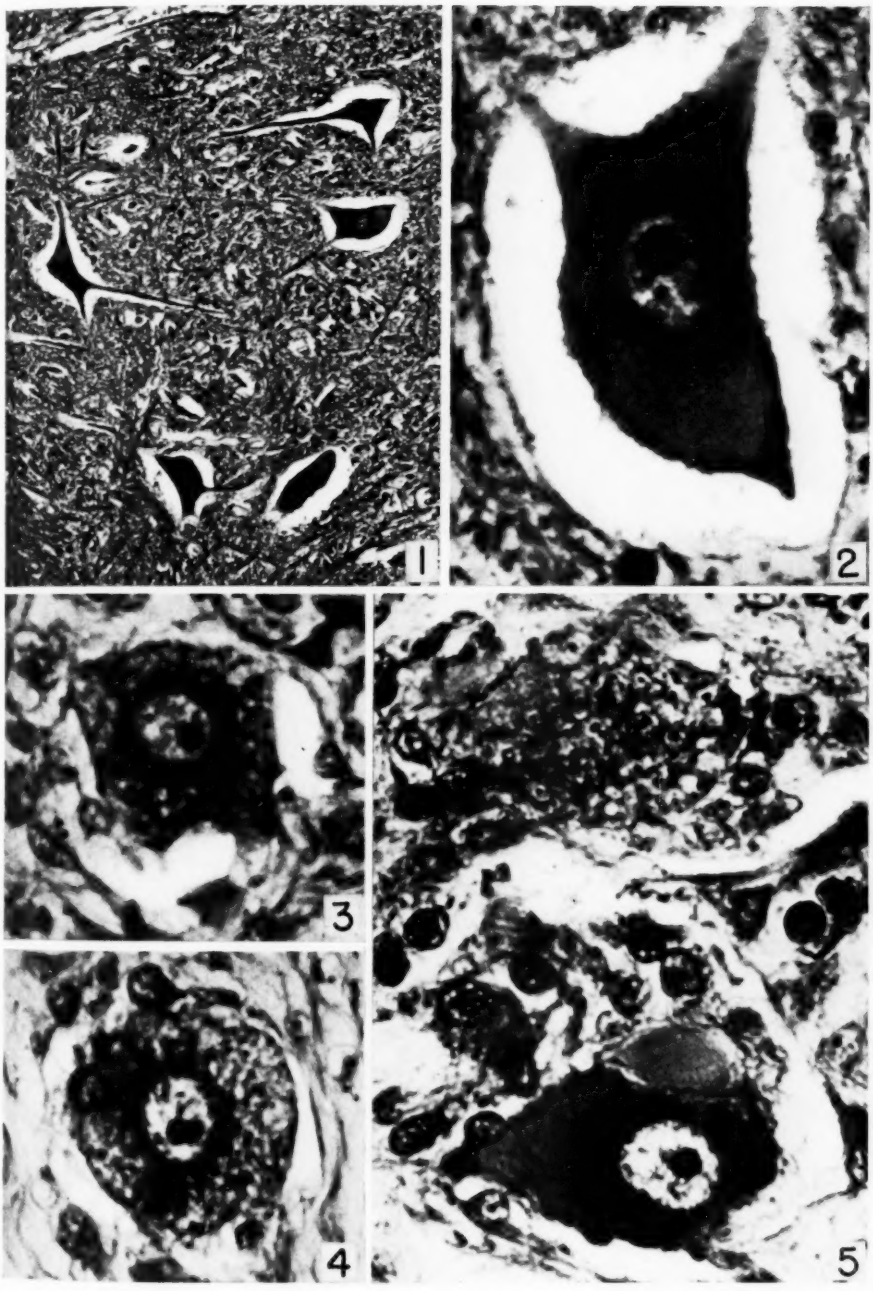
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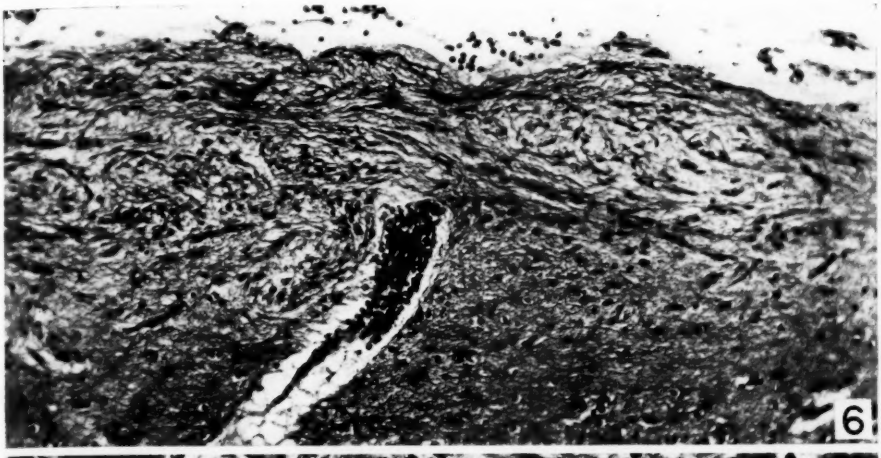
PLATE 86

FIG. 6. Perivascular cuffing in glomerular layer of olfactory bulb (case 1). × 160.

FIG. 7. Interstitial infiltration with mononuclear cells in gasserian ganglion (case 1). × 500.

FIG. 8. Focal cellular infiltration in abdominal sympathetic ganglion (case 1). × 670.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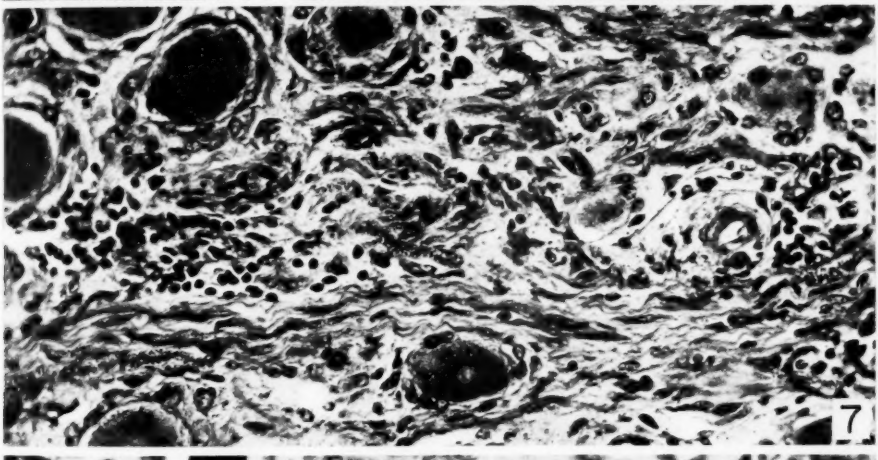
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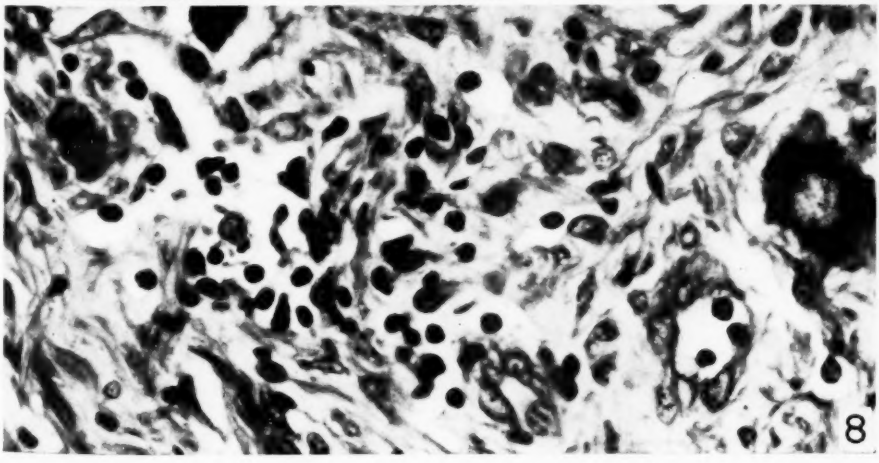
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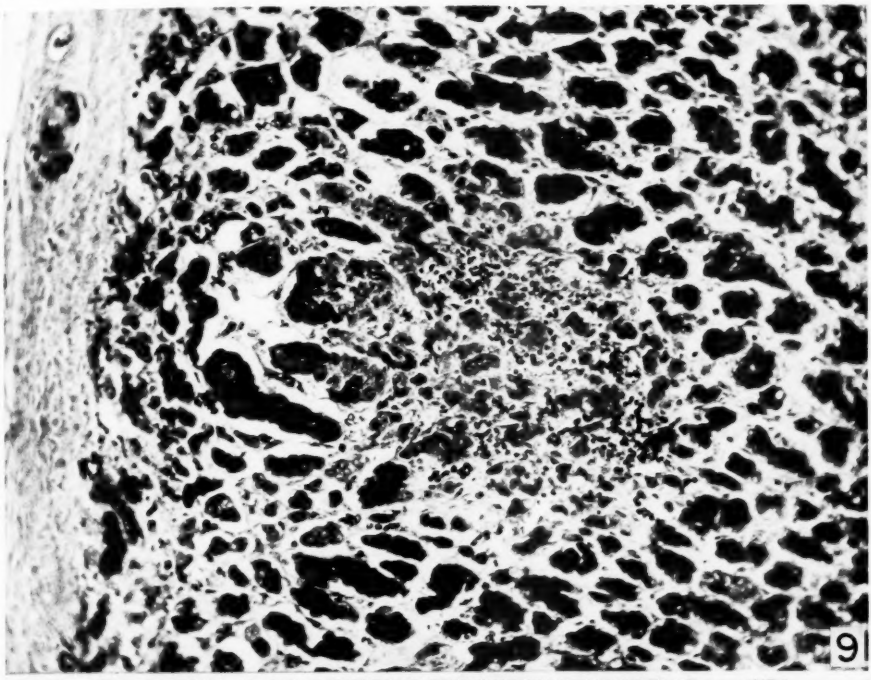
PLATE 87

FIG. 9. Adrenal (case 1): focal degeneration and cellular infiltration. × 160.

FIG. 10. Adrenal (case 1): cellular infiltration along nerve. × 140.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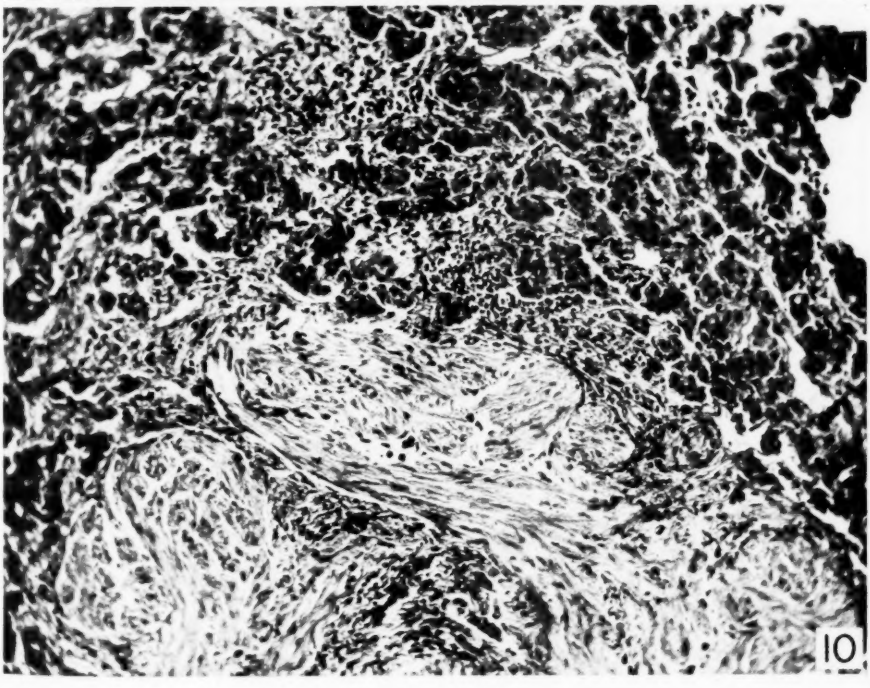
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PLATE 88

FIG. 11. Adrenal (case 3); interstitial cellular infiltration and vacuolization of some of the cortical cells. × 160.

FIG. 12. Adrenal (case 1); focal cellular infiltration. × 670.

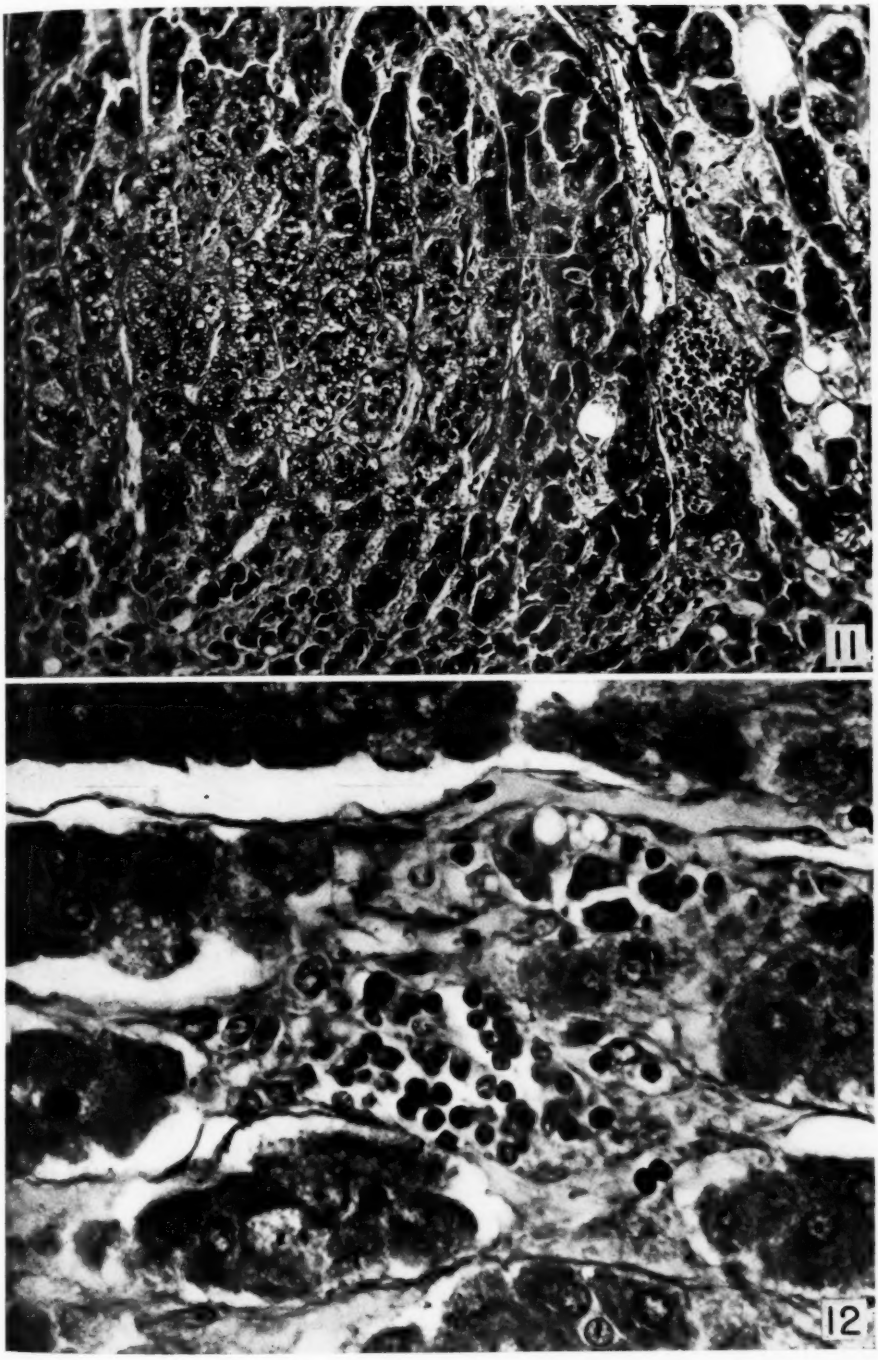
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PLATE 89

FIG. 13. Liver (case 1): cellular infiltration in Glisson's capsule. × 325.

FIG. 14. Liver (case 1): cellular infiltration in interlobular connective tissue. × 160.

FIG. 15. Liver (case 2): cellular infiltration in portal space. × 160.

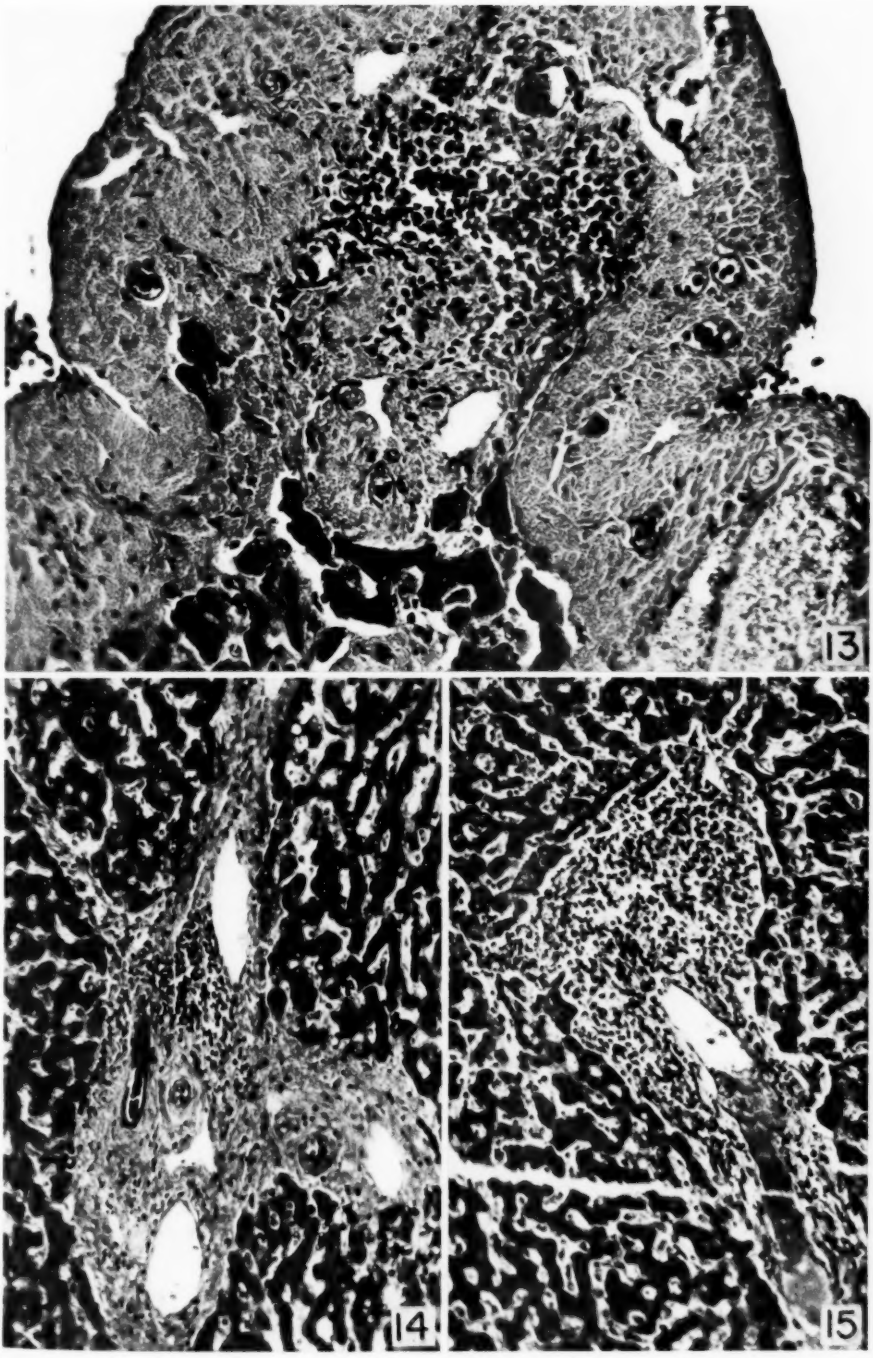
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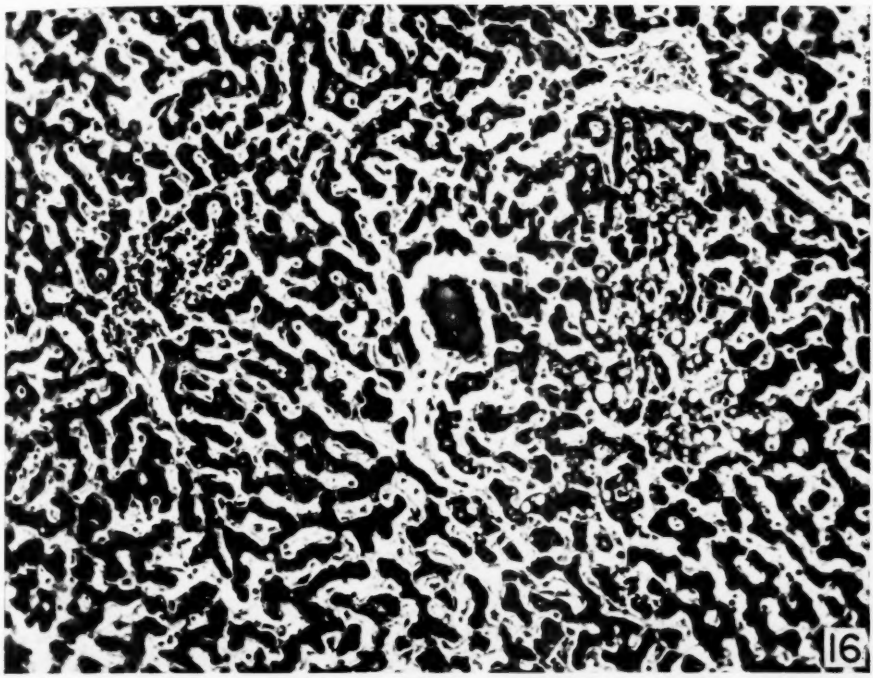
PLATE 90

FIG. 16. Liver (case 2); focal necrosis and cellular infiltration at left and fatty degeneration and infiltration at right. × 160.

FIG. 17. Liver (case 2); focal necrosis and cellular infiltration. × 670.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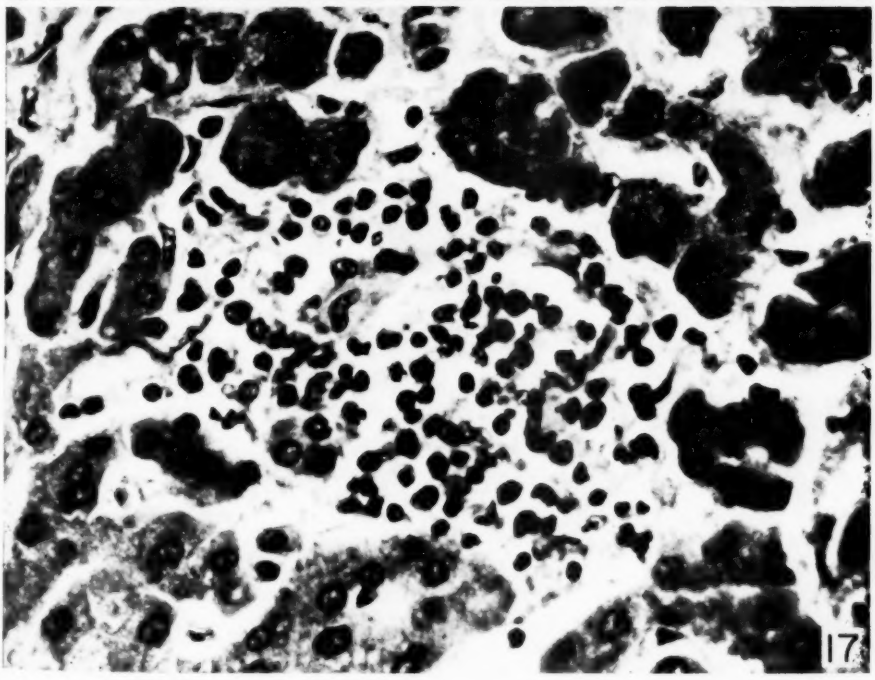
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PLATE 91

FIG. 18. Heart (case 2): interstitial cellular infiltration and phagocytic cells surrounding disintegrating muscle fiber at top. × 160.

FIG. 19. Heart (case 2): "myophagia"—phagocytic cells and disintegrating muscle fiber. × 1030.

FIG. 20. Heart (case 3): focal cellular infiltration. × 670.

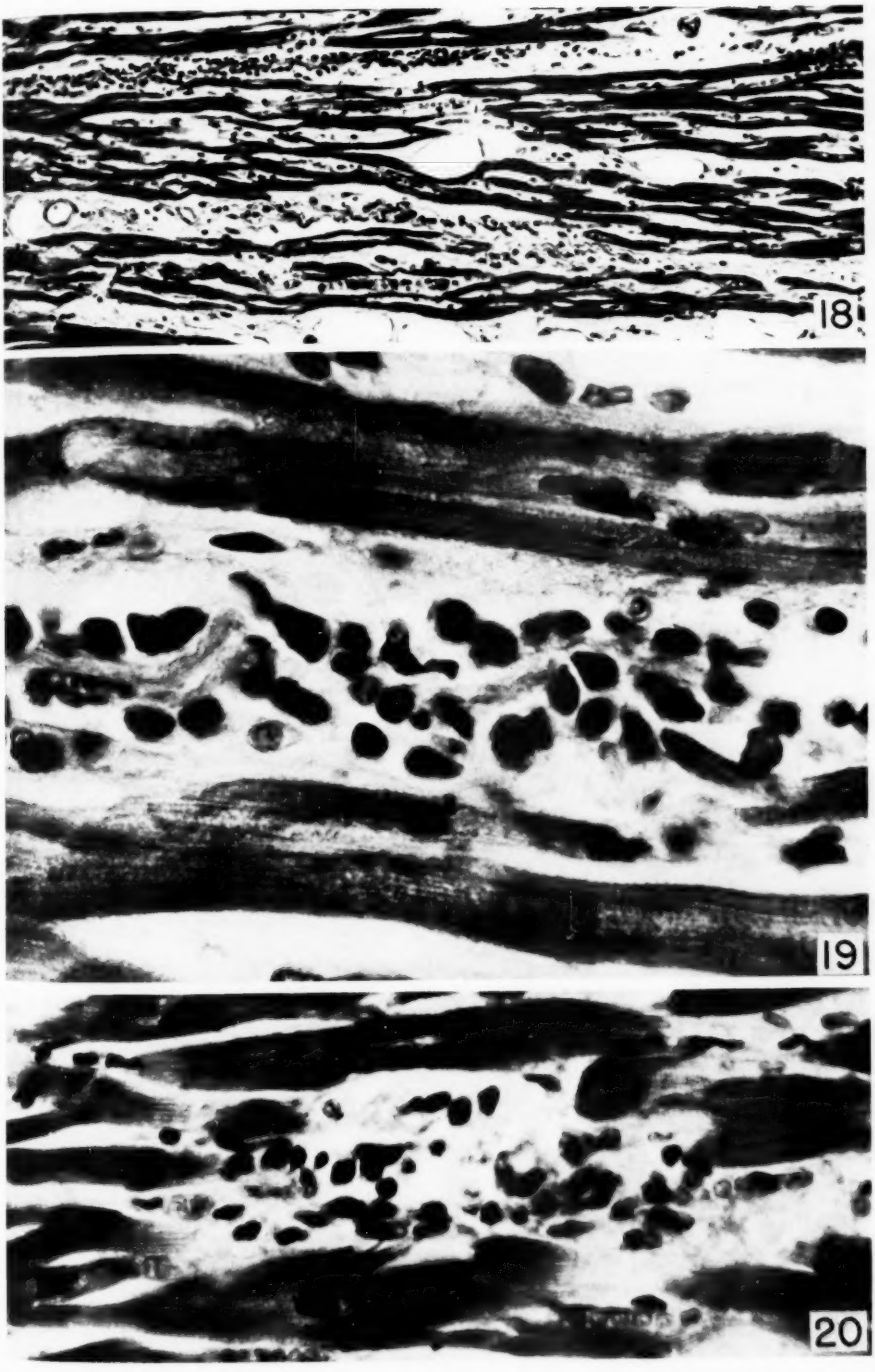
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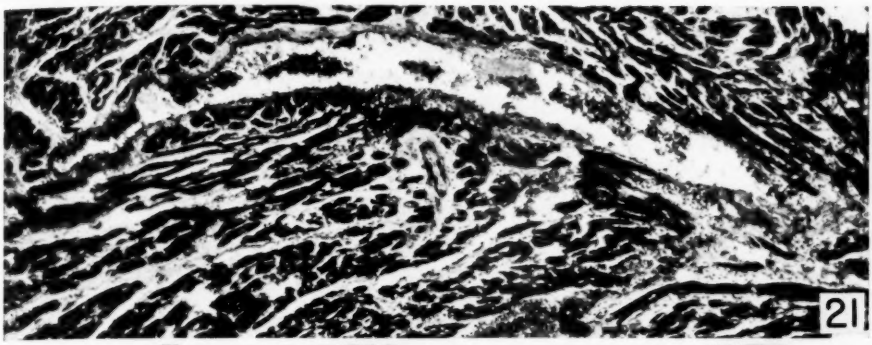
PLATE 92

FIG. 21. Heart (case 2); focal phlebitis and diffuse interstitial cellular infiltration. × 57.

FIG. 22. Heart (case 2); high power detail of wall of coronary vein showing edema and infiltration with polymorphonuclear and mononuclear cells. × 670.

FIG. 23. Kidney (case 2); intertubular cellular infiltration and congestion of adjacent vessel. × 160.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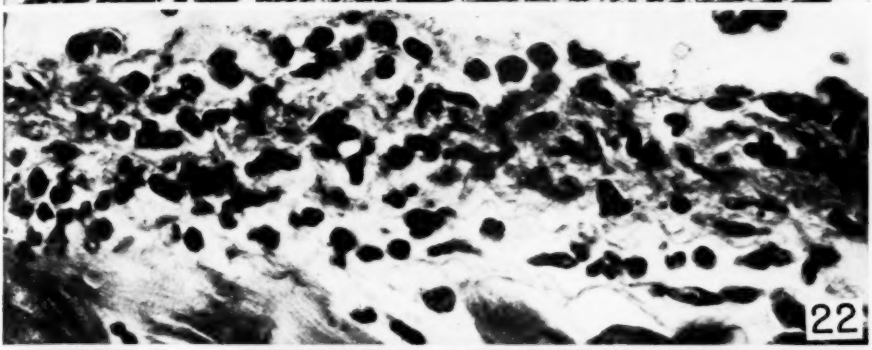
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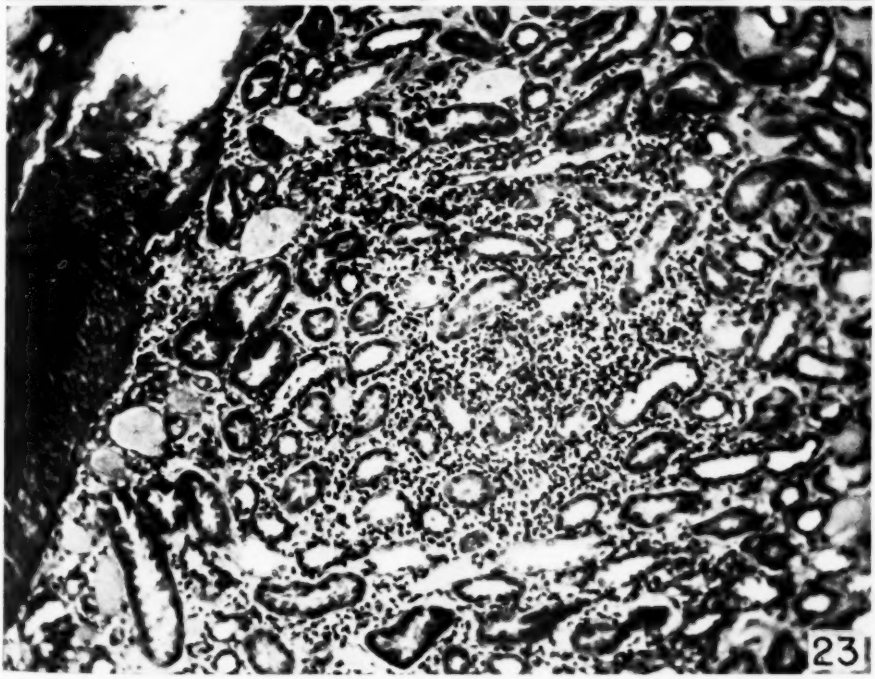
23

METASTATIC TUMORS OF THE MYOCARDIUM *

A Review of Sixteen Cases

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Metastatic tumors of the myocardium are relatively infrequent. In the autopsy series of the Wisconsin General Hospital, with slightly over 3,000 autopsies recorded at the time of writing, 16 cases had occurred.

Clinically, no sign of involvement of the heart muscle had been found even when extensive invasion had occurred. In every instance, any impairment of cardiac function noted during life was adequately explained by the non-neoplastic cardiac lesions present, such as coronary sclerosis, myocardial fibrosis or valvular lesions.

In 1935 Shelburne [1] reported a primary cardiac tumor diagnosed during life by reason of (1) a comparatively sudden onset of cardiac decompensation without known cause; (2) rapid accumulation of bloody pericardial fluid, which did not clot on standing; (3) lack of positive evidence of tuberculosis or syphilis, and (4) predominance of lymphocytes in the white cells of the pericardial fluid, eliminating the possibility of acute pericarditis. Heart block was also present. Such phenomena were absent in the present series.

Fishberg [2] recorded 3 cardiac tumors, diagnosed antemortem by the presence of auricular fibrillation in 2, and auricular flutter in the third. The only patient showing fibrillation in the present series (No. 30:194) had noticed an irregular heart beat "as long as she could remember"; little importance therefore can be attached to that phenomenon in this particular case.

Besides the myocardial metastases here reported, there were 23 cases with metastasis to the pericardium, representing 11 different types of primary tumors: carcinoma of the lung, 6 cases; lymphosarcoma, 5 cases; melanosarcoma, 3 cases; carcinoma of the stomach, 2 cases; and endothelioma, carcinoma of the renal pelvis, reticulo-endothelioma, carcinoma of the fundus uteri, car-

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TABLE I

Sixteen Cases of Metastatic Myocardial Neoplasia

Case No.	Type of tumor	Part of heart invaded	Route of invasion	Other lesions of heart	Other metastases
28:131 M., 66 yrs.	Rhabdomyosarcoma of kidney	Myocardium of right auricle	Blood-borne	Hypertrophy, fibrosis, slight coronary sclerosis	Adrenal, abdominal lymph nodes, liver, lung, ribs, vertebrae
30:50 F., 45 yrs.	Scirrhous carcinoma of breast	Subepicardial muscle of left ventricle	Blood-borne to epicardium, thence by lymphatics to myocardium	Atrophy, congestion and edema, hydropic degeneration, lipomatosis, slight fibrosis	Lymph nodes, liver, pleura, adrenal, uterus, pancreas, chest wall
30:193 M., 50 yrs.	Reticulo-endothelioma, primary in skin(?)	Atrioventricular groove; left ventricle; aortic valve	Blood-borne	Hypertrophy, diffuse fibrosis, lipomatosis, coronary sclerosis, edema	Lung, lymph nodes, pancreas, bladder, peritoneum, pelvic connective tissue, skin, voluntary muscle
30:194 F., 75 yrs.	Small spindle cell sarcoma of lung	Pericardium; muscle of left auricle	Direct extension	Coronary sclerosis, atrophy, fibrosis, dilatation	Bronchial nodes, by extension only
30:211 M., 29 yrs.	Melanosarcoma from mole on leg	All parts: all layers	Blood-borne	Diffuse fibrosis	Practically all organs except ureters
31:31 F., 49 yrs.	Carcinoma of bronchus, columnar-celled in some parts, squamous in others	Right ventricle (epicardium, muscle, and endocardium)	Blood-borne(?) to epicardium, thence by lymphatics to myocardium	Coronary sclerosis, edema, hypertrophy, perivascular fibrosis	Opposite lung, bronchial lymph nodes, liver, peritoneum, adrenals, uterus, ovary, mesentery
32:34 M., 57 yrs.	Reserve (or indifferent) cell carcinoma of lung	Right auricle (epicardium and myocardium)	Regional lymphatics (May have been direct extension)	Atrophy, in general; mitral stenosis; hypertrophy of right ventricle; congestion, edema, and round cell infiltration of muscle	Thyroid, pleura, ribs, kidney, lymph nodes
33:145 M., 31 yrs.	Adenocarcinoma of rectum	Right ventricle	Blood-borne to epicardium, thence by lymphatics to myocardium; tumor cells also in blood vessels of myocardium	Auricular thrombus	Pelvis, abdominal lymph nodes, lungs, parietal pleura, spleen, liver

Case No.	Type of tumor	Part of heart invaded	Route of invasion	Other lesions of heart	Other metastases
34:125 M., 46 yrs.	Prickle cell carcinoma of esophagus	Myocardium of left ventricle	Blood-borne	Thrombus in cardiac vein, endocardial thrombus, congestion, edema, fibrosis	Bronchi and mediastinum (extension)
36:23 M., 55 yrs.	Mesothelioma of pleura	Scattered throughout myocardium	(1) Blood-borne to myocardium; (2) blood-borne to epicardium, with direct extension to myocardium	Slight hypertrophy, coronary sclerosis, mitral sclerosis and fibrosis, myocardial fibrosis	Lungs, brain, kidneys, lymph nodes, skin, peritoneum, adrenals, pancreas
36:69 F., 57 yrs.	Adenocarcinoma of fundus uteri	Left ventricle, epicardium and muscle (cells in blood vessels)	Blood-borne to epicardium; tumor cells in blood and lymph vessels in myocardium	Congestion, slight fibrosis, cloudy swelling and hydropic degeneration	Lung, lymph nodes, liver, spleen, pancreas, adrenals, kidneys, gallbladder, peritoneum
36:291 M., 58 yrs.	Squamous cell carcinoma of lung	Left ventricle (epicardium and myocardium)	Blood-borne to epicardium, thence by lymphatics to muscle	Perivascular fibrosis	Bronchial and vertebral lymph nodes and adrenal; chest wall by extension
37:217 M., 69 yrs.	Myogenic sarcoma of bladder	Left ventricle, right auricle, all layers	Blood-borne	Sclerosis of aortic valve, coronary sclerosis, patchy fibrosis, endocardial thrombus	Esophagus, pleura, lungs, liver, spleen, pancreas, adrenals, ureters, colon, abdominal wall
38:111 M., 74 yrs.	Adenocarcinoma of pancreas	Left ventricle, muscle	Blood-borne	Coronary sclerosis, edema and patchy fibrosis	Gallbladder, mesentery, lungs
38:236 M., 45 yrs.	Lymphosarcoma (primary site undetermined)	(Autopsy done elsewhere; incomplete gross description)	Probably blood-borne to epicardium, with direct extension to muscle		Lung, diaphragm, intestine
39:41 M., 69 yrs.	Adenocarcinoma of pancreas	Right auricular appendage	Blood-borne	Auricular thrombus, hypertrophy, perivascular fibrosis	Lungs, lymph nodes, liver, peritoneum, adrenals, spine

cinoma of the thyroid, perithelial angiosarcoma of the pleura, and Hodgkin's endothelioma, each 1 case.

Table I summarizes the essential features of the 16 cases with myocardial metastases but certain items of special interest may be mentioned at greater length.

In case No. 30:194 a diagnosis of sarcoma of the lung was made. It was recognized that this resembled the tumor usually diagnosed as small cell or oat cell carcinoma, but the growth was massive and apparently metastasized only by direct extension. Further, a reticulum stain revealed reticular fibers in intimate contact with the tumor cells and forming processes which appeared to take their origin from the cells. On this basis the tumor was considered a sarcoma.

Case No. 34:125 illustrated a multiplicity of modes of spread, the bronchi and mediastinum being invaded by direct extension from the primary site in the esophagus, whereas the tumor cells in the myocardium very obviously came by way of the coronary arteries.

In case No. 32:34 there were two primary tumors: a carcinoma of the lung, the major malignancy which gave rise to the myocardial metastasis, and a carcinoma of the prostate which spread only by local extension to the seminal vesicles and bladder. The two tumors differed so widely in their histologic structure that there was no difficulty in determining the source of the metastases.

Case No. 39:41 presented a histologic picture of special interest. The wall of the right auricle was invaded by metastatic cells from a primary tumor in the body of the pancreas, and the right auricular appendage contained a decolorized thrombus. Within this thrombus was a cystic space of microscopic size, lined by cancer cells.

Case No. 36:23 introduced a controversial question, as many authorities doubt the existence of true mesothelioma of the pleura. The diagnosis in this case was made on the following gross and microscopic pathological considerations: the presence of a massive pleural growth which appeared, in the light of careful gross and microscopic scrutiny, to be invading the lung from the pleural surface; the lack of any other site which could be regarded as primary, even after diligent search; and the bizarre morphology of the cells, which in some fields formed sheets suggesting a cov-

ering tissue and in others were spindle-shaped, resembling cells derived from connective tissue. Undoubtedly many pathologists would call this tumor a carcinoma of the lung and it is impossible to prove that this was not a pulmonary tumor. It was felt, however, that the preponderance of evidence favored the conclusion drawn.

Discussion

An analysis of this series reveals the great variety in type and origin of the primary lesions; in 16 cases, 13 different types of primary tumors are represented. There were 3 examples of carcinoma of the lung and 2 of carcinoma of the pancreas but these were the only types to be repeated. This is in agreement with Yater,[3] who found in his exhaustive review of the literature that metastasis to the heart had occurred from neoplasms of all the main organs.

On further examination it appears that 7 of these 16 primary tumors were located in or about the chest. This observation could logically be anticipated, since such tumors are usually in fairly close proximity to the heart. It indicates a possibility of regional lymphatic dissemination which should be borne in mind when the clinical course suggests cardiac metastasis.

The degree of general spread of the tumors in this series is of interest. In 10 of the 16 cases the distribution of metastases could be considered as generalized. On the other hand, in one case (No. 34:125), a carcinoma of the esophagus, the myocardium was the site of the only remote metastasis, the bronchi and mediastinum being invaded by direct extension. Such an occurrence is infrequent. Burke,[4] for instance, found in his series of 14 cases that the heart was never the sole site of metastasis. Yater [3] made no specific observation on this point.

Three routes of invasion, as outlined by both Yater [3] and Burke,[4] are recognized; namely, the blood stream, the lymphatics and direct extension, lymphatic invasion being from the mediastinal lymph nodes against the lymph stream. In the present group, all three modes have occurred. A combination of routes was present in several cases, involving the transport of malignant cells through the blood stream to the epicardium and progression thence into the muscle through the lymphatics or by direct extension. In certain cases in which extensive invasion had occurred,

it was impossible to determine with certainty the route of metastasis by examination of microscopic sections. For example, in case No. 30:50 the epicardial nodule was so diffuse that it was impossible to ascertain by the microscopic appearance whether the tumor cells were blood-borne or carried through the lymphatics. A review of the gross description, however, revealed that there was an isolated epicardial nodule near the apex of the left ventricle. This fact, added to the presence of metastases in such remote organs as the uterus and adrenal, led to the conclusion that this was a blood-stream invasion of the epicardium.

Again, in a few cases malignant cells were seen in both the blood vessels and lymphatics of the myocardium. Whether the cells in the two vascular systems had been transported independently, or whether this invasion represented the rupture of tumor cells into vessels within the myocardium, could not be determined with certainty.

The modes of growth within the muscle, as distinct from the routes of metastasis, were those characteristic of the various types of tumors and took three general forms: (1) invasion through the channels, such as the lymphatics and blood vessels; (2) infiltrative invasion with varying degrees of myocardial destruction; and (3) massive growth (microscopically speaking), with displacement and complete destruction of muscle fibers.

No primary tumors of the heart were found in the autopsy series from this laboratory.

Consideration of the ages of the patients reveals nothing of particular interest, as in general they correspond to the age groups in which these types of tumors are apt to be found.

Twelve out of the 16 patients were males. This appears to represent a great predominance in the male sex, but such a conclusion is modified when one considers that in 857 cases of malignancy in the autopsy series studied there were 594 males and 263 females, a proportion not far from that found in the series of myocardial metastases.

Summary

Sixteen cases of metastatic tumors of the myocardium are reported, with a tabulation of certain features and a brief discussion. Thirteen different types of primary tumors were repre-

sented, and there was considerable variation as to route of metastasis and mode of growth within the muscle. In no case had a clinical diagnosis of cardiac invasion been made.

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LOBULAR CARCINOMA IN SITU *

A Rare Form of Mammary Cancer

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(From the Pathological Laboratories of the Memorial Hospital, New York, N.Y.)

With increasing emphasis on the early diagnosis of cancer it is obvious that the pathologist will observe increasingly early histologic manifestations of this disease. In recent years it has become apparent to us that various forms of carcinoma *in situ* were being encountered in ever greater frequency and in locations where such phenomena were hitherto but rarely discovered. Examples of entirely noninfiltrative lesions of a definitely cancerous cytology have been accumulated for almost every mucosa-lined structure.

Carcinoma *in situ* in the breast is a disease which has been recognized for many years. The term, however, has not been employed and for the usual form of the disease the designation "noninfiltrative comedo-carcinoma" has served. Nevertheless, comedo-carcinoma constitutes an example *par excellence* of carcinoma *in situ* of a glandular organ. This form, however, is a disease mainly of the larger duct system. One is much less apt to think of carcinoma *in situ* as a disease of small lobular ducts and lobules. The latter process is relatively rare. One of us (F. W. S.) had occasion several months ago to conduct a clinical-pathological symposium † on tumors of the breast at which a lesion of this type was presented. It was found that the malignant character of the process was not recognized by a number of pathologists. For this reason it is felt desirable to review certain features of such tumors.

An impression of the incidence of this type may be gained through a survey of the mammary cancers observed during the past year at the Memorial Hospital. There were two typical examples of strict carcinoma *in situ* of lobules and terminal lobular ducts in approximately 300 primary, operable, mammary cancers. Additional examples of the lesion have been seen during

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† For the American Society of Clinical Pathologists.

this period in material submitted from other institutions for consultation. The latter fact testifies to nonrecognition of the fundamental potentialities of the pattern.

It is apparent, however, that this type of mammary cancer, *i.e.*, cancer originating in lobules and terminal ducts, is more common than this incidence would appear to indicate, for, when the tumor infiltrates, it is apt to do so in a peculiar fashion which permits one, after some experience, to recognize the high probability of such origin even though it is impossible actually to trace it. Moreover, in the fully infiltrative form it is often possible to detect outlying areas where lobular carcinomatosis *in situ* is still very apparent. Thus, in these same 300 cases there were 5 in which the pattern was very marked, 2 in which it was moderately developed, and 5 in which it was noted definitely but was scanty in amount.

When this pattern of carcinogenesis is present it is not necessarily the only mode of origin. This may be true, but in some instances a lobular type is combined with different modes of development. Thus there are patterns of combined lobular carcinoma and infiltrating duct cancer beginning in multiple papillary adenomatosis, lobular cancer combined with quite dissimilar large cell comedo-carcinoma both infiltrating and *in situ,* and lobular carcinoma plus tubular adenocarcinoma—again quite dissimilar histologically. Hence it is wholly improbable that this lobular carcinoma *in situ,* or "totilobular carcinoma," as we have occasionally designated it to emphasize its origin in terminal ducts and all constituents of the lobule, constitutes a separate entity other than in the clinical sense and when in its noninfiltrative phase. At that stage involvement of lymph nodes has never been seen.

There is no way in which a clinical diagnosis of lobular carcinoma *in situ* can be made. Patients with cancers of this variety, or with the later infiltrative phase, are in the same age group as are those bearing other mammary cancers. In the noninfiltrative phase the breast reveals none of the classic clinical signs of cancer. The nipple is erect. Retraction is absent. Skin dimpling and fixation are absent. Discharge or bleeding from the nipple has not been noted. The mass is movable and diagnosis is usually "chronic cystic mastitis" or fibro-adenoma. In fact, in our most

marked case the surgeon encountered a gush of fluid on exploration of the mass, a number of small cysts were noted grossly, and cancer remained wholly unsuspected until sections were made. There is no way by which it *can* be recognized grossly. The pathologist sees only congeries of what look like large lobules, if indeed he recognizes anything at all abnormal. Since there is little piling up of epithelium in terminal ducts, necrosis does not occur and hence the chalky streaks so characteristic of many cancers are lacking. Of course, with the development of infiltration the gross morphology becomes that of any mammary cancer. The existence of unrecognizable lesions of this totilobular structure is disquieting to one who trusts his gross diagnosis and suggests the necessity of frozen section in any lesion where disproportion in the size of lobules is evident.

Microscopically the process shows the following characters: There is a sudden and abrupt alteration in lobular cytology (Fig. 1). A group of normal-appearing lobules is interrupted by the presence of a lobule or group of lobules in which, although these lobules may be within normal limits in size or even smaller than normal, the cells are large (Fig. 2). They are perhaps twice the size of those of the normal lobules and their nuclei are in proportion. The nuclei tend to be rather clear; they show no hyperchromatism. The cytoplasm is apt to be opaque, somewhat acidophilic, and occasionally vacuolated. The compact, orderly arrangement of the epithelium of the normal lobule gives place to a decided looseness, a loss of cohesion. Layers do not multiply as layers but cells are progressively displaced toward the lumina in a disorderly fashion, eventually obliterating the space. Slight degenerative changes may result in the formation of central mucoid globules. Mitoses are rare. It is usually necessary to survey a section of two or three entire lobules to find a single mitosis. The cells lose polarity, varying in shape while maintaining surprisingly uniform size. They occasionally assume what looks like a loose reticular structure.

The type of lobule which undergoes this transformation varies. Large lobules, small lobules, lobules with mucoid stroma, metaplastic lobules, and hyalinized lobules, may all assume this pattern. Occasionally only part of a lobule is involved and a sharp line of division between normal epithelium and carcinoma *in situ*

is seen. No constant perilobular inflammatory infiltration accompanies the change.

The earliest manifestation of lobular carcinoma *in situ* may be found in isolated cells or groups of cells in the lobule or in the terminal lobular duct (Fig. 3). Presumably multiple isolated cells are the first sign but that stage is soon past. Such cells recall certain features of Paget's disease and we have designated them "pagetoid" cells. The clinical entity, Paget's disease, has not been encountered in this group of cases. In some areas there is a suggestion of general lobular epithelial hypertrophy prior to the stage which one might designate as neoplastic, a graded progressive increase in cell size so gradual that demarcation is impossible. In the earliest phase, the occurrence of pagetoid cells is limited to those cells near the limiting membrane.

It should be emphasized that this lesion occurs in multiple lobules. It has been forcibly impressed upon us that a breast in which this process occurs in the slightest degree constitutes an extreme hazard. Whereas it is not clinical cancer until infiltration occurs, it is always a disease of multiple foci. Hence it is never safe to leave the breast with local excision only, even if the entire palpable lesion has been removed. Whenever the process has been found by local excision, subsequent simple mastectomy has shown additional foci of disease. In our first case, local excision revealed this process and we were unfortunately not aware of its significance. Within the space of a few months the patient had infiltrating cancer with axillary metastases and now has skeletal dissemination. It is our feeling that simple mastectomy is essential, with further procedure dependent on finding the least evidence of infiltration.

The mode of infiltration of these lobular cancers is peculiar and somewhat obscure. One often sees evidence of a sudden, almost explosive liberation of cells from their natural boundaries. The term "explosive" is used with full realization that temporal elements are not known. Nevertheless the resultant picture is often that of a terminal duct, possibly showing the noninfiltrative phase of the tumor, but surrounded by large numbers of isolated, loose cells of rather uniform size but of varying shape (Fig. 4). They are not especially hyperchromatic. In some fields they might readily be confused with large mast cells. In others

they suggest the morphology of the periductal myoid cells. They are, however, liberated cancer cells and when they metastasize to nodes their form and distribution are such that they might be confused with cells of reticulum cell sarcoma. Their wide infiltration within the breast itself may lead to the invasion of residual lobular connective tissue in lobules which themselves have not given rise to neoplasm. Thus they replace lobules which undergo atrophy. Why atrophy occurs is not know to us. Pressure in this case does not satisfactorily account for it although it may very well do so in the case of expanding mammary cancers of more solid type. The isolated cells may evoke considerable desmoplastic response on the part of the connective tissues.

Since distension of the lobule does not assume marked proportions prior to infiltration, some other factor must be invoked to explain the mass eruption of tumor cells. We suspect some lytic action of the tumor cells, naturally not to be detected by anatomic study.

The question has arisen as to whether tumors of this type deserve the designation of "acinar" carcinoma. We have not as yet observed a mammary cancer which in our opinion might properly be considered acinar cancer. It is largely a question of terminology. We do not feel that we can draw sharp lines between terminal ducts and acini. We prefer to regard the acinus as a structure which develops during lactation and which thus constitutes a physiologic phase rather than an anatomic entity. It is to avoid confusion that we employ the term "lobular carcinosis *in situ*."

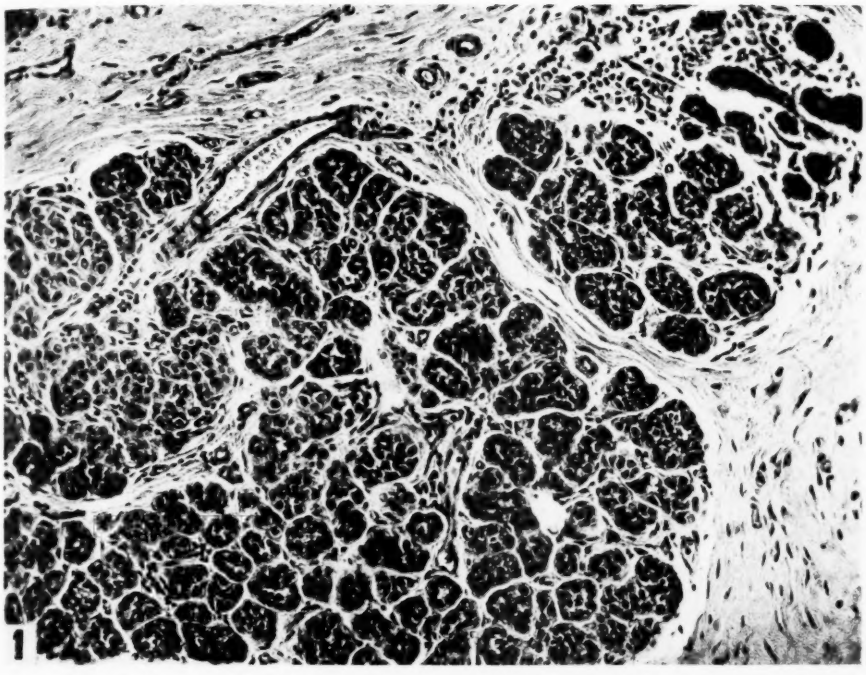
Naturally, the existence of lobular carcinoma *in situ* presupposes that lobules are present. The breast may not be atrophic. Nevertheless there exists a type of carcinoma which apparently takes origin in smaller ducts and which begins in cells of pagetoid type, identical with those seen in lobular carcinoma *in situ*, and this type of cancer may occur in the non-lobule-containing atrophic breast.

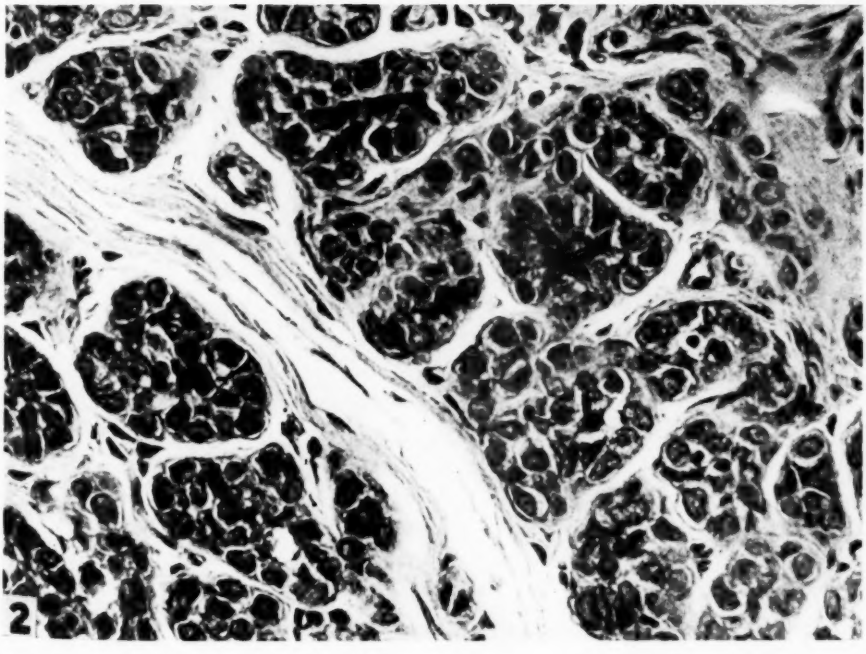
DESCRIPTION OF PLATES

PLATE 93

FIG. 1. Lobular carcinoma *in situ*. At the upper right there is shown a portion of a normal lobule for comparison. × 170.

FIG. 2. Higher magnification of involved lobules in the same area as Figure 1. × 450.



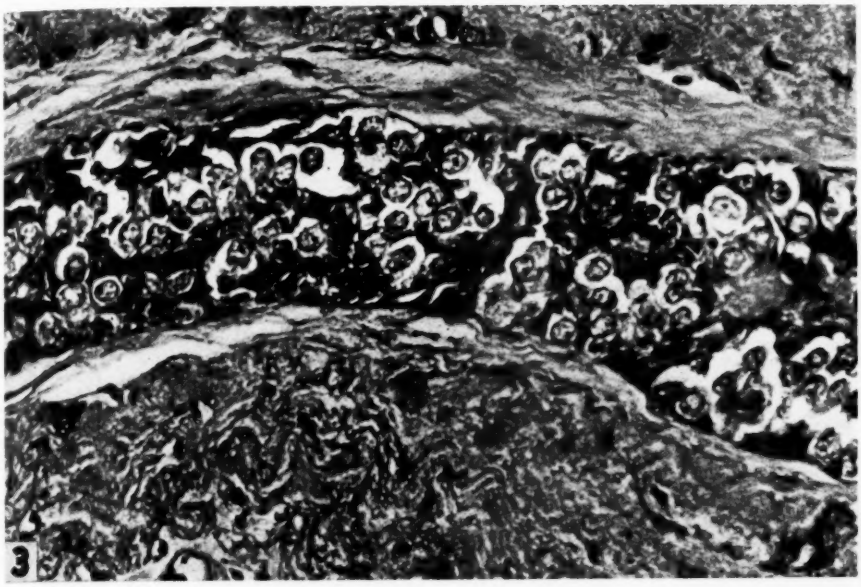


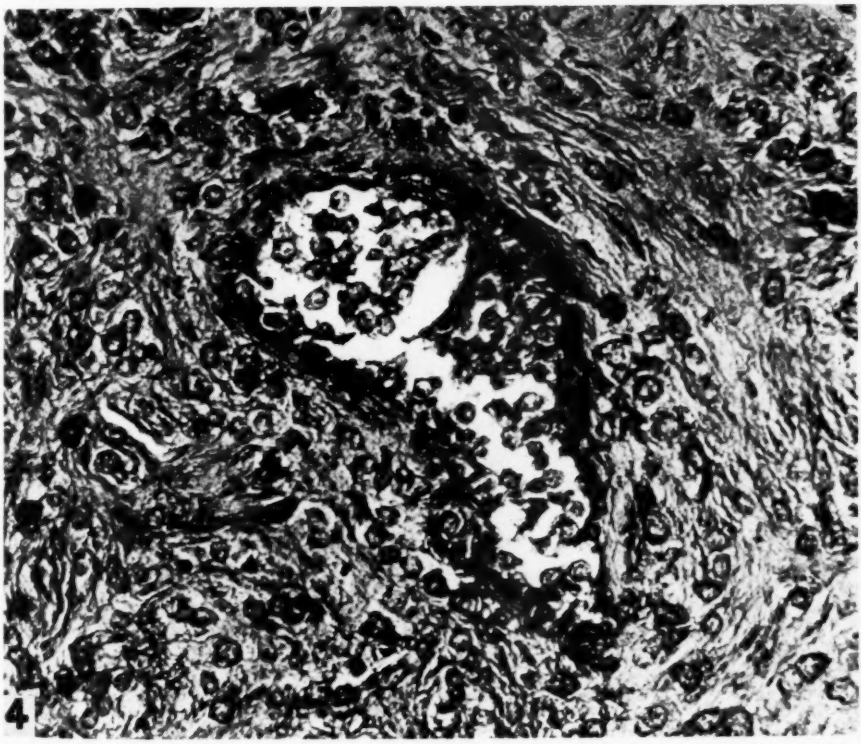
Foote and Stewart Lobular Carcinoma *in Situ*

PLATE 94

FIG. 3. Terminal duct with "pagetoid" cells. × 450.

FIG. 4. Invasive phase developing from lobular carcinoma *in situ*. A terminal duct with "pagetoid" cells is shown, with surrounding infiltrative cancer cells. Despite the infiltration the periphery of the lobule (not shown) still showed connective tissue encapsulation. × 450.





Foote and Stewart Lobular Carcinoma *in Situ*

HISTOGENESIS OF EWING'S TUMOR *

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The origin of Ewing's tumor of bone from vascular endothelium [1, 2] is a view that has not been uniformly accepted. Connor [3] adhered at least in part to the endothelial origin in his first communication on the subject but later [4] modified this opinion in favor of the reticulo-endothelial derivation, and added that the cell of Ewing's sarcoma might assume osteoblastic properties. Oberling [5] and later Oberling and Raileanu,[6] after studying a group of Ewing tumors by special staining methods, concluded that the tumors were derived from a primitive mesenchymal cell, multipotent and capable of differentiation toward endothelium, reticulo-endothelium or into blood-forming elements. Geschickter and Copeland [7] have suggested that the cell concerned may be the lymphoblast. Roome and Delaney [8] advanced the possibility that a myeloid stem cell (hemocytoblast) is the active cellular element. In a criticism of possible sources for development of Ewing's tumor, Melnick [9] asserted that this tumor is neither endothelioma, reticulo-endothelial sarcoma nor myeloma, but is a round cell sarcoma springing from undifferentiated embryonic mesenchymal cells situated in connective tissue about blood vessels in the haversian canals. De Santo [10] has recorded a case which he believed supported the view that Ewing's tumor originated in lymphatic endothelium of the haversian canals and was hence a lymphangio-endothelioma. All of these authors at least accept the existence of a disease commonly called Ewing's tumor of bone and yet Ewing's tumor as an entity has been questioned by Colville and Willis [11] and again by Willis.[12]

One of the great difficulties encountered in studying the origin of certain tumors lies in the unfortunate manner in which these tumors occur. Primary growth patterns are frequently obliterated and little or no opportunity for observing their mode of progression remains. In the case of Ewing's tumor, this is eminently true. This group is characterized by relatively long duration of symp-

* Received for publication November 25, 1940.

toms before diagnosis is made and accordingly the process is usually fully developed when recognized. To complicate further the picture from the standpoint of histopathological interpretation, the bulk of material is made unsatisfactory by such factors as scanty biopsy and previous irradiation. Occasionally, however, a particularly suitable specimen is obtained from which certain decisive observations can be made. Recently, we have seen a case which we believe reveals something of the fundamental growth characters of Ewing's tumor.

Report of Case

Clinical History. The patient, D. D., a boy 15 years of age, was admitted to the Memorial Hospital, Johnstown, Pennsylvania, on May 20, 1940 with the chief complaint of pain in the right chest for 8 years, dyspnea on exertion and pain in the lumbar region for 3 weeks. He gave a history of having had typhoid fever 8 years before, at which time several transfusions were required for epistaxis. Since then he had complained of pain in the right chest, aggravated by exertion. This pain had been intermittent in character. Three months before admission he had an attack of "quinsy" and since had had continuous chest pain. In addition, for 3 weeks prior to admission, there had been pain in the lower lumbar region. Examination of the thorax revealed the left chest to be normal to percussion and auscultation. Expansion was slightly impaired on the right. The percussion note was slightly impaired anteriorly, flat in the axilla and impaired posteriorly from the fifth rib down. Breath sounds were distant or absent in the lower part of the right chest. Vocal resonance was diminished slightly throughout the right lower lobe, but not to the same degree as the breath sounds. Occasional fine, crackling râles were present in the right base posteriorly.

Roentgenologic Examination. Radiographs revealed an irregular ovoid, soft tissue mass which appeared to originate at the right diaphragm and which extended up to the level of the inner end of the right third rib. This mass was rather homogeneous and it was thought that it might contain fluid. Moreover, it was considered to be intrapulmonary rather than in the interlobar fissure. The possibilities of a thick-walled intrapulmonary cyst, a primary diaphragmatic tumor or interlobar abscess were advanced. On the day after admission, a fluoroscopic examination of the chest was done and the mass in the right lower lobe was found to be free from the diaphragm and not to move with diaphragmatic excursion.

An aspiration biopsy was suggested 3 days after admission. Films of the chest showed no change in the primary lesion but at this time scattered, possibly exudative lesions were noted throughout the right lung as well as throughout the lower two thirds of the left lung. At this time attention was called to an involvement of the right seventh, eighth and ninth ribs in the midaxillary line, consisting of what was thought to be a periosteal thickening or periostitis together with a moderate amount of localized bone condensation. This process involved particularly the seventh rib. Two possibilities were considered at this time: a primary tumor originating in the rib and

extending in an intrapulmonary direction, and, because of the history, an interlobar abscess, which, by direct extension, had involved the ribs.

On June 5, a bronchogram with lipiodol was done. This showed an elliptical shadow which seemed to follow the lower interlobar fissure and on the basis of this finding a thoracotomy with biopsy was urged.

Surgical Procedure. Operation was done on June 25. Under cyclopropane anesthesia, a curved incision was made parallel to the right seventh rib, the center being in the midaxillary line. The periosteum of the rib was much thickened. The intercostal nerve was necrotic as a result of either osteomyelitis or tumor. About 3 in. of the seventh rib were removed. On attempting to locate the parietal pleura, it was found that what seemed to be tumor had involved this structure and extended to the periphery of the lung in that region.

Histology

The microscopic diagnosis of the tissue removed at operation was Ewing's endothelial myeloma of bone. The pertinent histological features are illustrated in the photomicrographs appended, all being taken from an area of bone involvement. These fields lay within an area not exceeding 1 cm. in diameter. In Figure 1 is shown what we believe to be the basic pattern of endothelial myeloma. Here, the tumor retains at least a vestige of organoid character, this being represented by formed vascular spaces lined by elongated cells which closely resemble endothelial cells and lie in a relationship expected of this cell. These cells are not entirely uniform, as might be expected. There is some variability in size and shape and some are more heavily stained than others. At one point, enclosed in a vascular channel, is a mitotic figure, poorly shown in the illustration, being slightly out of focus with the remainder of the field. These channels do not contain erythrocytes or leukocytes and hence it cannot be determined whether they are related to lymphatics or to blood vessels. Careful survey of this immediate area fails to reveal the presence of any actively participating cell that might belong to the hemopoietic series, nor do the cells under consideration resemble reticuloendothelial cells. The proliferative qualities of these cells are readily shown by the gradual coalescence of groups of single cells to form small masses in which no vascular channels are seen. The structure is more than a little suggestive of areas that may be seen in hemangioma hypertrophicum cutis or in some cellular subcutaneous lymphangiomas. The chief importance of Figure 1 is that it demonstrates clearly the vasoformative properties re-

tained by the tumor. This is a necessary attribute of any tumor if proof of origin from vascular endothelium is to be obtained, a point that has been stressed by Melnick.[9] In Figure 2, vasoformative properties are not preserved and the only resemblance to endothelium which remains is the persistent spindle or elliptical shape of some of the cells. Diffuse overgrowth has now occurred and fully developed diffuse endothelioma is pictured. This is the structure most commonly seen in Ewing's tumor with the exception that the individual cells are more spindle-shaped and elliptical. The preponderance of this growth type in these tumors is responsible for Ewing's [1, 2] term, "diffuse endothelioma."

The occurrence of cells in rosette arrangement is another property often noted in Ewing tumors, though by no means peculiar to this tumor. A group of these structures is seen in Figure 3. They are not numerous in this case. The individual cell type here is that usually encountered in Ewing tumors. Other features present, but not illustrated here, included areas of reactive new-bone formation at the advancing edge of the tumor. The occurrence of this new-bone formation was not seen at every peripheral area. Instead, several areas showed clearly the spread of the tumor into the marrow spaces and haversian canals with no concomitant proliferation of osteoblasts. In the sites of bone formation we were unable to trace transition stages from the tumor cells to osteoblasts. We do not believe that the cells giving rise to Ewing's tumor have any osteoblastic capabilities, inasmuch as bone formation does not occur in the visceral or lymph node metastases of this tumor. Bone formation is reactive and accompanies periosteal elevation.

In the case presented it is difficult to escape certain conclusions. Definite vasoformative properties are shown and the structure seen in Figure 1 is scarcely one that could be duplicated by multiple myeloma, lymphosarcoma, metastatic neuroblastoma or metastatic bronchogenic carcinoma, the tumors commonly mentioned as causing confusion with Ewing's endothelial myeloma of bone. It is not possible to determine whether this particular tumor is derived from lymphatic endothelium or blood-vascular endothelium. Ewing has formerly favored perivascular lymphatic endothelium as the probable site of origin, having traced one of his early cases [2] to this location. At the present

time, he is less restricted in his view and believes that lymphatic or blood-vascular endothelium may be concerned. The ultimate cell type he now designates as "vascular endothelium possessing angioblastic properties" and for some time he has employed the term "capillary angiosarcoma."

Ewing [13] has also commented on the obscurity of any etiological factor in endothelial myeloma and has called attention to a possible relationship to chronic osteomyelitis. In the present case, it is of interest to note that for 8 years, following typhoid fever, this patient complained of pain in the right chest, suggesting the possibility that typhoid osteomyelitis may have been present and that an endothelial tumor may have arisen in the granulation tissue.

Summary

A case of Ewing's endothelial myeloma of bone is presented that is clearly traceable to vascular endothelium. The structure shown by this tumor is regarded as wholly inconsistent with anything known in multiple myeloma, reticulo-endothelial sarcoma, lymphosarcoma, metastatic carcinoma or metastatic neuroblastoma. The thesis of origin of Ewing's tumor in vascular endothelium is sustained.

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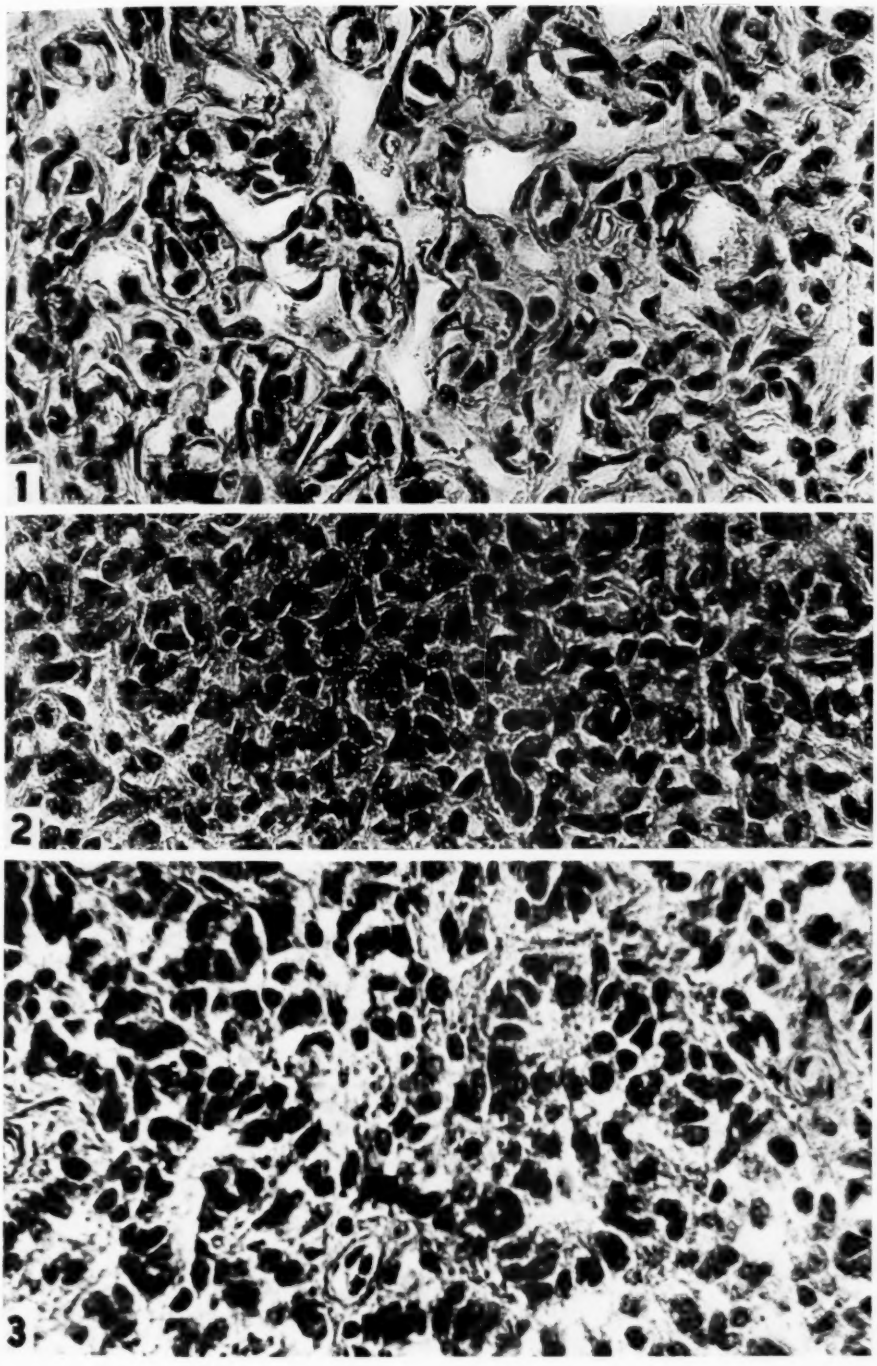
DESCRIPTION OF PLATE

PLATE 95

FIG. 1. Basic, capillary, angiomatous, vasoformative structure of Ewing's tumor. × 500. (The three figures on this plate were photographed from closely adjacent areas of the same neoplasm.)

FIG. 2. Transition to diffuse endothelioma without total loss of resemblance to endothelial cells. × 500.

FIG. 3. Cells in rosette arrangement. × 500.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ENDOTHELIAL MYELOMA (EWING'S TUMOR OF BONE) *

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In 1933 Colville and Willis [1] concluded that the subject of Ewing's tumor of bone was in a chaotic state and that the occurrence of a primary growth of bone of this nature was still unproven. These authors expressed the belief that metastatic tumors of bone of various types, especially neuroblastoma, will probably prove responsible for many cases. As to the case in question the conclusion was reached that the tumor had been primary in the adrenal, although a tumor of the femur appeared to have been extremely extensive, widely disseminated metastases were present in many bones and viscera, and tumor nodules were present in *both* adrenals. In fact, the cut surface of the right adrenal, as illustrated, showed a score of small nodules, both cortical and medullary, the size of the organ was not definitely abnormal, and the pathological picture failed to correspond to that of the commonly recognized adrenal neuroblastoma, since it is usually impossible in fatal disease of this type to be able at autopsy to recognize any adrenal at all. The supposed neuro-epithelial rosettes failed to reveal fibrillary processes with appropriate silver stains.

In a more recent report Willis [2] reached an essentially similar conclusion, this time despite the fact that the femoral tumor had existed for 3 years. He believed the primary tumor to have been retroperitoneal but failed to show how this mass was to be distinguished from metastases in adjacent lymph nodes. He likewise seemed to accept the neuro-epithelial nature of the rosettes although the only stains capable of offering proof of this contention apparently were not done. Willis asked how rosettes can be characteristic of "endothelial myeloma" when they are commonly present in neuroblastoma, evidently assuming that the term "characteristic" must mean that the feature may never be possessed by more than one thing. He dismissed the 5-year cures of Ewing's tumor of bone as unconvincing and apparently was willing to assign them to confusion with osteomyelitis or syphilis

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of bone. As a matter of fact, the "cured" cases of Ewing tumors of bone, all with pathologic material, in the collection of the Registry of Bone Sarcomas of the American College of Surgeons, were recently reviewed by Stewart [3] and eleven were accepted as examples of this disease. The longest "cure" was of 31 years.

That metastatic neurocytoma can produce the radiographic picture of Ewing's tumor of bone cannot be doubted. In the Memorial Hospital series this is extremely common in retinocytoma. Almost every case of terminal, highly malignant retinocytoma will yield multiple bone metastases, most commonly subperiosteal and especially near epiphyseal lines, less often medullary. Such metastases are radiographically indistinguishable from Ewing's tumor.

It is true that there are few cases of Ewing tumor of bone with adequate autopsy study. For this reason the following apparently typical case is recorded.

Report of Case

The patient was a male, 14 years of age. He was admitted to the hospital on March 13, 1940. Two months prior to admission he had sustained an injury while playing. Immediately thereafter a painful swelling of the thigh was noted. Continuous fever developed, the tumor of the thigh continued to increase in size and 3 days prior to admission it broke down and discharged sanguinous fluid.

The patient was wasted, anemic, and very ill. The thigh was enormously swollen. The tumor was globular in shape and measured, by external palpation, 76 cm. in maximum circumference, 43 cm. in length and 28 cm. in thickness. It was tender and a discharging wound was present. The condition was obviously a terminal one. No further investigations were made and the patient died 2 days later of terminal pneumonia.

Gross Findings

Complete autopsy was performed. The primary tumor was situated in the femur. It measured 48 cm. in maximum circumference. The cut surface measured 33 by 18 cm. The major portion of the mass was grayish, cystic and necrotic. A few areas, particularly at the marginal portions of the tumor, were whitish and appeared to be very cellular. Here the tissue was intact. The bone showed two pathologic fractures. The cortical portion of the shaft appeared necrotic and rarefied; the medulla appeared structureless. Save for the fact that the tumor surrounded the femur and had caused double patho-

logic fractures, it would not have been possible to state whether it arose in bone or in adjacent soft tissues. Postmortem radiographs (Fig. 1) were reported as showing general decalcification of bone with worm-eaten structure, double pathologic fractures, very little evidence of reaction or repair and a large soft tissue extension of tumor. The appearance was regarded as consistent either with metastatic disease in bone or a rapidly growing primary osteolytic bone tumor.

The brain, abdominal viscera, thoracic viscera other than the right lung, and the soft tissues generally, showed no evidence of tumor. The adrenals were carefully investigated, with negative findings. The only tumor apart from the main mass consisted of a small nodule 1 cm. in diameter on the external surface of the base of the right lung. Aside from bilateral terminal pneumonic consolidation, no other lesions were demonstrated.

Histology of the Bone Tumor

The most prominent histological feature was the presence of a large number of pseudorosettes (Fig. 2). Between the pseudorosettes was seen a diffuse growth of uniform polyhedral cells. In places an occasional capillary blood vessel was surrounded by similar cells—the characteristic perithelial arrangement. One peculiar feature worth emphasizing was the lack of intercellular connecting fibers, the cells being quite separate from one another. The individual cells were uniform in size and shape. They appeared polyhedral. The nuclei were not hyperchromatic. There were no mitotic figures to be seen. The nuclear material took a homogeneous stain and the cytoplasm was very scanty. The histology was consistent with the diagnosis of endothelial myeloma of bone. Sections were stained for reticulum fibers by Foot's method and it was found that the cells of the tumor had no specific relation with the reticulum fibers. A few discrete reticulum fibers were seen.

Histogenesis

The histogenesis of Ewing's tumor is still under active debate. Oberling [4] was the first to bring forward evidence that the growth is in reality a reticulosarcoma. According to him there are several histological types. The first has the structure of an undif-

ferentiated or syncytial reticulosarcoma. In the second there is some degree of differentiation with formation of reticulin fibrils. The third is frankly dictyocytic and the fourth lymphoblastic. Ewing [5] has recently expressed himself as follows:

"Oberling and Raileanu [6] and others have presented evidence to show that this tumor arises from the reticulo-endothelial system and that the tumor cells exhibit capacity to differentiate into plasma cells, myelocytes, lymphocytes and even erythroblasts. According to this view, the tumor represents a form of totipotent myeloma capable of forming any one of the specific types of myeloma. This interpretation seems to have been rather widely accepted in Europe. The writer cannot accept this view and believes that the eminent French investigators have failed to distinguish between a specific type of endothelial tumor and other rarer round cell myelomas among which may probably be some that arise from the hemopoietic cell system or indifferent reticulum cells. The writer believes that the typical endothelioma of bone arises from capillary endothelium and never exhibits any other properties than those belonging to vascular endothelium. The pseudorosettes, the characteristic perithelial structures, and the cords of polyhedral cells lining elongated spaces are the outstanding structural features of this tumor and they never appear in any tumor derived from hemopoietic cells or reticulum cells. Plasma cells, granular leucocytes and lymphocytes are notably absent from tumors presenting these features, and when they are present the tumor should be excluded from the group of endothelioma. Moreover, in many cases of endothelioma there are associated with the above structural features, dilated blood channels of various sizes composed of the typical tumor cells, disclosing the angioblastic properties of the cells and connecting the tumor with other angiomas or angiosarcomas. Similar features are observed in capillary angiosarcomas of other organs, notably the skin."

The histology of the present case is consistent with Ewing's interpretation.

Discussion

There are various features worth emphasizing in this case: (1) origin in the femur, the commonest site of Ewing's tumor, as reported by Geschickter and Copeland [7] (incidence 28 per cent); (2) the short clinical duration of only 2 months; (3) the history of trauma at the onset which obviously brought to notice an already existing disease of the bone; (4) the solitary metastatic lesion despite large size of the primary mass; (5) the presence of

a fracture at two places, an unusual feature in Ewing's tumor; (6) an histological picture very unusual in respect to the large number of pseudorosettes in a single field.

SUMMARY

A case of Ewing's tumor of bone is recorded in which complete autopsy revealed a single small pulmonary metastasis and nothing whatever which could be regarded as a primary neuroblastoma. Doubt, recently expressed, concerning the existence of the entity "Ewing's tumor of bone" is considered untenable.

NOTE: Grateful acknowledgment is made to S. R. Joglekar and P. V. Gharpure for the use of their material.

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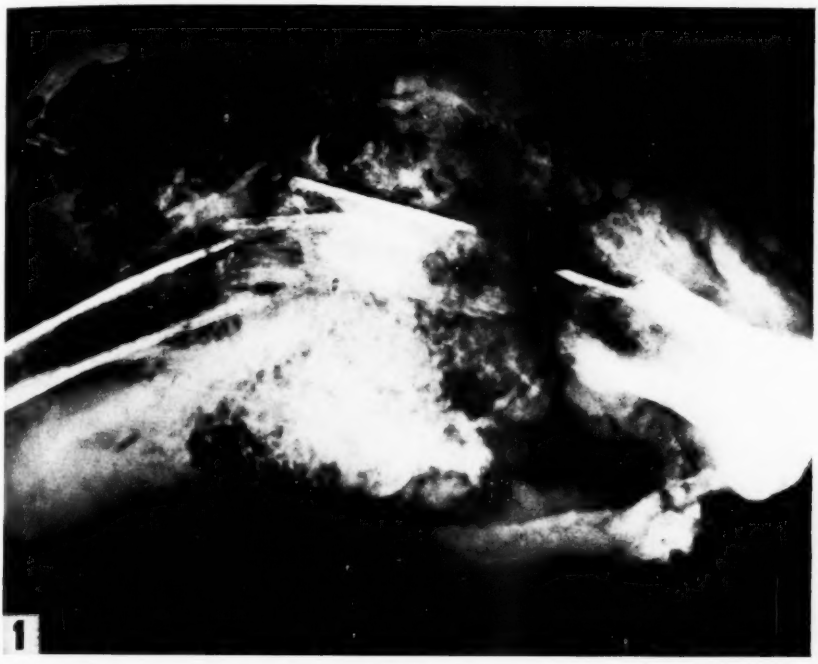
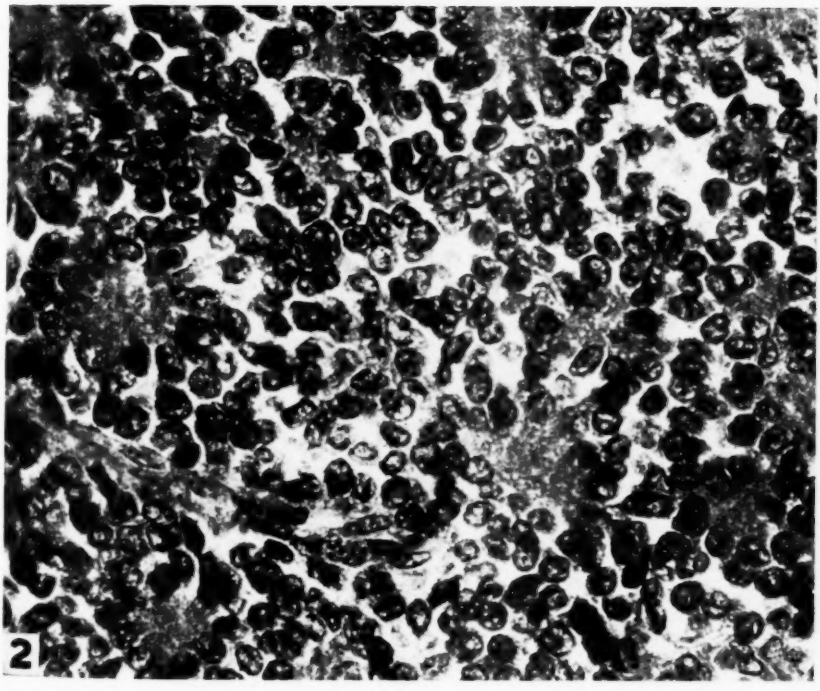
DESCRIPTION OF PLATE

PLATE 96

FIG. 1. Roentgenogram, made after autopsy, showing a double fracture of the femur produced by a Ewing's tumor.

FIG. 2. Several closely approximated pseudorosettes in Ewing's tumor of bone. × 350.





Gharpure Endothelial Myeloma

EFFECT OF THE PITUITARY GROWTH HORMONE ON THE EPIPHYSEAL DISK OF THE TIBIA OF THE RAT *

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INTRODUCTION

Recently Freud, Levie and Kroon [1] have suggested testing the potency of growth hormone preparations by means of tail growth and vertebral development in hypophysectomized rats. This method offers definite advantages both in simplicity and reliability over body weight increments which usually show considerable variation. However, before a biological method of assay such as this can be generally accepted, the influence of both intrinsic and extrinsic factors affecting the response must be established. Does the age of the animal have any influence? Is the response confined to the epiphyseal cartilage as claimed by Freud, Levie and Kroon? If so, do all the epiphyseal cartilages of the body react in the same manner? Furthermore, what is the effect of other endocrines, particularly those commonly found in alkaline extracts of the anterior pituitary, on the skeleton? Do any of these synergize or antagonize the action of growth hormone? This preliminary investigation deals with the first of these questions, the purpose being to determine the influence of *age* on the response of the skeleton to growth hormone injections in both unoperated and hypophysectomized rats.

A specific relation of pituitary derangements to skeletal growth has been suspected clinically for some time. In 1921, Evans and Long [2] first demonstrated the effects of anterior pituitary extracts on body weight as a whole. Dott and Fraser [3] in 1923 showed a definite skeletal relation experimentally in a study on dogs and cats. Later, Handelsman and Gordon,[4] using the skull and mandible of the rat as "test bones," studied age differences in response to growth hormone injections and reported that animals under 90 days of age showed little or no stimulation of bone growth if the

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period of injection was less than 3 weeks, while "in rats weighing about 190 to 250 grams, the potency of an extract can be evaluated in as short a time as two weeks." On the other hand, Silberberg,[5] studying the endochondral ossification of the tibias of normal young guinea pigs (130 to 220 gm.), reported that after only 4 injections of acid extract of bovine anterior pituitary,* stimulation of cartilage and bone growth could be noted. Injections over a longer period of time caused a premature calcification and hence closure of the epiphyseal line. More recently, Freud, Levie and Kroon [1] have generalized their findings on the vertebrae, tibias and ribs and have stated that hypophysectomy in rats results in epiphyseal closure but that growth hormone injections, when instituted immediately following the operation, prevent this closure.

With the apparent contradictions in the reports on the response of the skeletons of rats and guinea pigs and because age is known to influence skeletal response in other disorders (*i.e.*, rickets), it was believed that this problem warranted further investigation.

Material and Method of Preparation

Forty female rats have been studied. These were divided into three age groups: approximately 54, 88 and 150 days of age at autopsy.

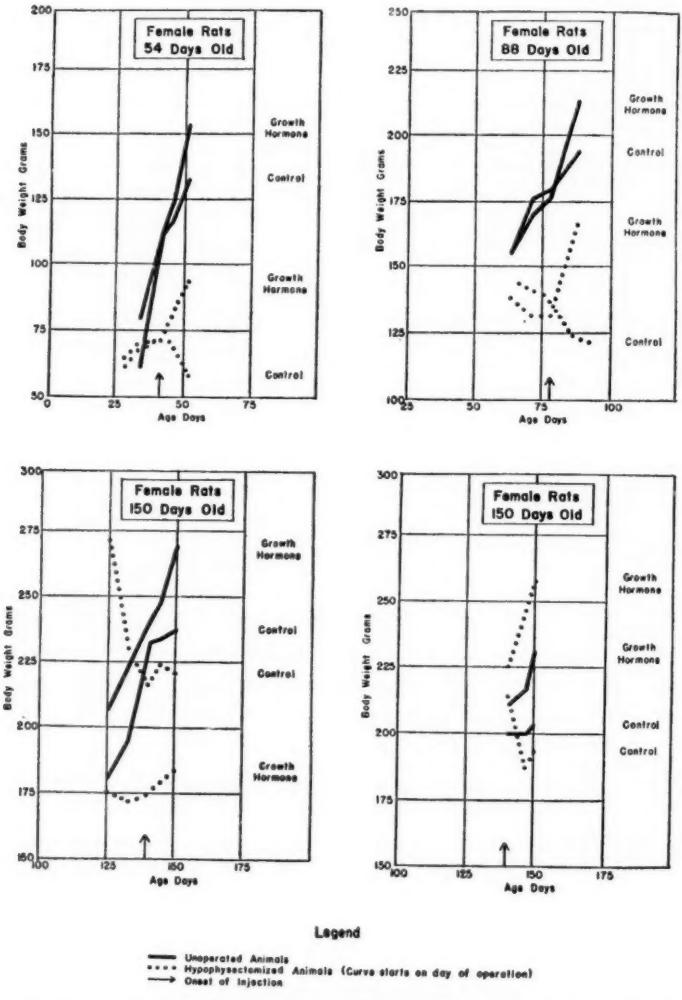
Each of these groups was subdivided into unoperated controls, unoperated injected animals, hypophysectomized controls, and hypophysectomized injected rats (Table I, groups A, B, C and D respectively). Females were chosen in order to eliminate sex factors and as far as possible litter mates were used in each age group. Following weaning, the animals were placed on a diet (Evans, No. XIV) consisting of 68 per cent ground whole wheat, 10 per cent fish meal, 10 per cent alfalfa leaf meal, 6 per cent casein, 5 per cent fish oil and 1 per cent NaCl. This diet has been tested on a series of 1100 females and 500 males and proved in this colony to result in growth comparable to that obtained on Diet 1, McCollum stock. At 28, 64, 125 and 140 days of age, respectively, half the animals in each group, chosen at random, were hypophysectomized by the parapharyngeal approach of

* It might be noted that the accepted method of extracting growth hormone is from an alkaline solution.

TABLE I
Distribution of Materials

Group	Age (*days*)	Post-operative days	Rat No.	Injection	
				No. of days	Total solid (*mg.*)
A. Unoperated controls	54		W 30		
	54		BH55		
	54		W 82		
B. Unoperated injected	54		W 29	10	14.4
	54		G 34	10	14.4
	54		W 53	10	14.4
C. Hypophysectomized controls	54	25	G 32		
	54	25	W 79		
D. Hypophysectomized injected	54	25	G 33	10	8.8
	54	25	W 51	10	8.8
	48	19	GH84	4	1.4
A. Unoperated controls	88		BH09		
	88		BH77		
			BH94		
B. Unoperated injected	88		BH08	10	68.7
	88		W 72	10	68.7
			BH92	10	68.7
C. Hypophysectomized controls	88	25	W 75		
	92	25	BH05		
	92	25	BH33		
	92	25	BH43		
D. Hypophysectomized injected	88	25	W 03	10	41.9
	88	25	W 73	10	41.9
			W 90	10	41.9
A. Unoperated controls	150		W 97		
	150		W 07		
	150		B 15		
	150		W 23		
B. Unoperated injected	150		B 53	10	84.2
	150		W 09	10	68.7
	150		W 24	10	68.7
	150		B 44	10	68.7
C. Hypophysectomized controls	150	25	W 88		
	150	11	B 27		
	150	11	B 14		
	150	11	B 42		
D. Hypophysectomized injected	150	25	W 58	10	54.7
	150	11	G 10	10	41.9
	150	11	B 16	10	41.9
	150	11	BH45	10	41.9

Smith.[6] The weight changes were followed for 2 weeks and if incompleteness of the operation was suspected, the animals were discarded. Completeness of hypophysectomy was further verified at autopsy. Following this 2-week period, the animals to be treated were given 10 daily intraperitoneal injections of growth



TEXT-FIGURE 1. Average body weights plotted against the age in days. Curves for hypophysectomized animals start on the day of operation.

hormone. The animals 140 days of age at hypophysectomy were injected on the day of operation. The foregoing data are summarized in Table I.

The growth hormone was prepared by Uyei [7] and had previously been standardized so that "acute" injections could be given, *i.e.*, double the amount of hormone necessary to give maximal growth. The total amount of hormone in milligrams of solid material injected during the 10-day period is also given in Table I.

The weight response was marked in the hypophysectomized animals of each age group (Text-Fig. 1). In the unoperated rats, this response varied considerably with the age, the difference between control and injected rats being greatest in the oldest group, least in the youngest. The curves in Text-Figure 1 represent the average body weights of the animals in each group as plotted at 5-day intervals during the experimental period. The increases in weight were not always constant so the figures for each animal have been included in Tables III to V ("Gain in body weight").

The right tibia of each animal was decalcified, embedded in nitrocellulose and sectioned serially at 8 to 9 μ. Three staining procedures were employed: Böhmer's hematoxylin and eosin, Mallory's azan, and a new stain developed by Koneff [8] for differentiating hyaline cartilage, calcified cartilage, preosseous substance and mature bone. Measurements made with an eyepiece micrometer are given in arbitrary units. Later the ocular was

TABLE II

Normal Rats: Summary of Measurements

Total number of rats	Age	Average body weight	Tibia, proximal portion		
			Cortical bone width*	Articular cartilage width*	Epiphyseal cartilage width†
	(*days*)	(*gm.*)			
1	24	47	13	23	127
1	28	61	12	17	102
3	54	135	15	13	38
1	74	188	28	14	63
3	88	196	20	15	31
2	90	240	22	15	28
1	100	186	23	15	48
1	123	194	26	13	38
4	150	212	25	11	40
1	170	213	26	15	45
1	305	250	22	10	27
1	384	250	25	14	17
1	406	268	20	14	23

* Measured with a calibrated ocular. One division is equivalent to 16.1 μ.
† One division equals 4.1 μ.

calibrated with a stage micrometer. The cortical bone (periosteal ossification) was measured in the proximal region of the diaphysis next to the fibula, the articular cartilage (hyaline cartilage proliferation) in the center of the articular surface, and the epiphyseal line (endochondral ossification) in the central portion.

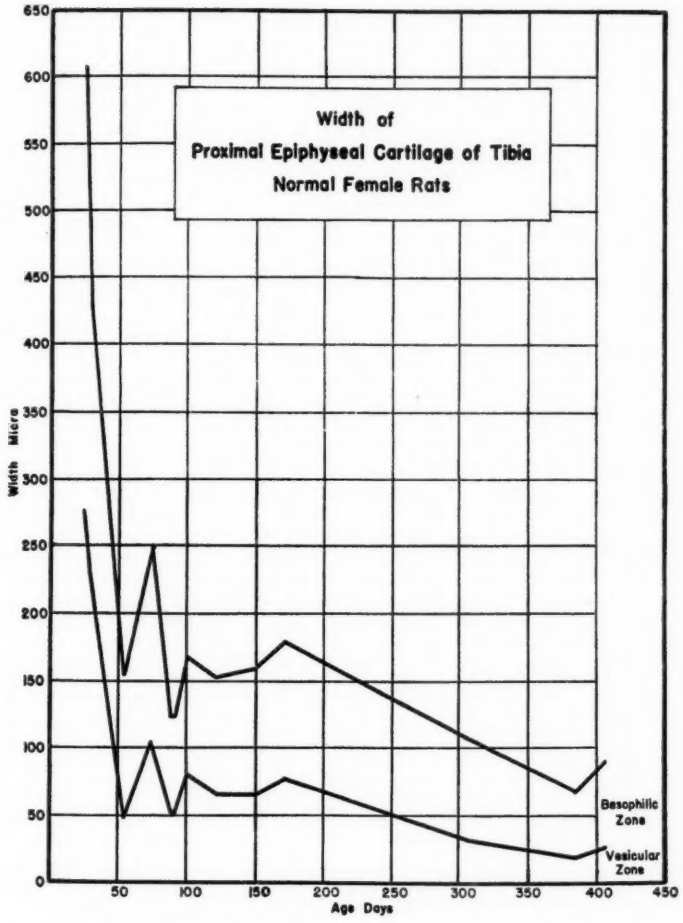
In addition to the rats already mentioned, serial sections were made also of the proximal portion of the right tibias of an additional group of normal animals (Table II). In these, every 20th section was photographed and a definite area in the tibia determined for all subsequent comparisons. This area in the group 54 days old was in the central portion of the lateral articular surface just medial to the fibula; in the other two groups it was in the central portion of the tibia between the two articular surfaces.

Results

In the young rat the epiphyseal disk may be divided into four definite zones, from the epiphysis toward the shaft:

1. Remnants of the embryonic hyaline cartilage with the cells irregularly arranged (Fig. 1, a).
2. Proliferating basophilic cells arranged in columns parallel with the long axis of the shaft (Fig. 1, b).
3. Vesicular cells, occupying large lacunae, also arranged in columns (Fig. 1, c).
4. Line of "erosion" where capillary loops from the vascular bed of the diaphysis meet the advancing rows of cartilage cells (Fig. 1, d).

Early in the life of a rat (between 24 and 54 days of age) an equilibrium is established in the tibia between the proliferation of cartilage and endochondral ossification at the epiphysis. This is shown graphically in Text-Figure 2. During this early period the original embryonic cartilage is rapidly replaced by bone and marrow until all that remains is the epiphyseal disk as found in the adult. From this period on there is a gradual reduction in the width of both the basophilic and vesicular zones (Text-Fig. 2 and Table II) but the proximal epiphyseal disk of the tibia persists until senility (Dawson [9]). A balance between deposition and resorption is also established in the cortical bone before 74 days of age and in the articular cartilage prior to 30 days of age



TEXT-FIGURE 2. Variation in the width of the epiphyseal cartilage with age.

(Table II). The establishment of these equilibria does not mean that skeletal growth ceases but that ossification keeps pace with cartilage formation and that remodeling accompanies periosteal ossification.

From Text-Figure 2 the age groups chosen for this experiment can readily be placed. The youngest group, 54 days of age, comes at the end of the period during which the epiphyseal plate of the

tibia is established. The middle group, 88 days of age, represents active but mature bone growth. The oldest group, 150 days of age, is representative of the suspended growth of the adult rat.

Histologically, the equilibrium established in endochondral ossification is less stable and hence more sensitive than that in periosteal and endosteal ossification. The embryonic hyaline cartilage is present as a continuous zone up to 50 days. At 88 days of age it is absent in places and by 150 days only scattered traces remain. Mitotic figures are frequent in the basophilic cells of the young rat but in animals 150 days old they were not observed. With advancing age there is a reduction in the length of the columns, and an increasing irregularity in their arrangement as well as an increase in the irregularity of the width of the disk as a whole (Fig. 2). These changes are accompanied by a decrease in the size and number of the individual cells and a relative increase in the amount of matrix (Figs. 6, 10 and 14). With the decrease in width of the vesicular zone (Text-Fig. 2) there is also a reduction in the size of the cells; the nuclei become somewhat more basophilic, and the columnar arrangement is less regular. Activity along the zone of erosion decreases with age and in old animals (150 days and over) bone is deposited along this region, thus effectively sealing the epiphyseal cartilage from the diaphysis. In young growing rats, however, longitudinal trabeculae of cartilage protrude into the medullary cavity of the shaft as growth proceeds and the surfaces become covered with a thin layer of preosseous substance. More distal in the diaphysis, the cartilage is gradually replaced by preosseous substance and finally by fully formed bone.

To summarize the histological changes in the epiphyseal disk of the normal rat tibia, with increasing age one observes:

1. Increasing irregularity in total width of disk.
2. Disappearance of embryonic hyaline cartilage.
3. Increasing irregularity in arrangement of cells in basophilic and vesicular zones.
4. Reduction in size and number of cells in both of these zones.
5. Lack of activity and subsequent deposition of bone along the line of erosion.

RAT TIBIA AT 54 DAYS

Effect of Growth Hormone on Unoperated Rats

The measurements of the various regions of the tibias of the unoperated rats, aged 54 days, are presented in Table III. In comparing groups A and B, the cortical bone and articular cartilage showed no changes in thickness following 10 injections of growth hormone. A slight increase in width of the epiphyseal cartilage was not great enough to be significant.

TABLE III

Rats 54 Days Old: Summary of Measurements

Group	Rat No.	Gain in body weight, 10 days	Tibia, proximal portion		
			Cortical bone width*	Articular cartilage width*	Epiphyseal cartilage width†
		(gm.)			
A. Unoperated controls	W30	30	17	12	40
	BH55	8	15	15	32
	W82	28	15	12	43
Average		22	16	13	38
B. Unoperated injected	W29	38	12	15	45
	GH34	34	17	14	44
	W53	34	12	13	36
Average		32	14	14	42
C. Hypophysectomized controls	G32	− 2	13	15	27
	W79	−16	11	..	14
Average		− 9	12	15	20
D. Hypophysectomized injected	G33	20	15	13	90
	W51	24	14	14	74
	GH84	5	14	16	55
Average		16	14	14	73

* Measured with a calibrated ocular. One division is equivalent to 16.1 μ.
† One division equals 4.1 μ.

The survey pictures showed a gross similarity between the control and injected rats. However, at higher magnification (Figs. 10 and 11) a definite increase in activity along the zone of erosion, following the injections, can be discerned. There is also a reduction in the size of the trabeculae with an increase in their number, and a slight decrease in the number of fat cells of the marrow.

Effect of Hypophysectomy

After a postoperative period of 25 days there was no significant change in width of the cortical bone or of the articular cartilage.

However, the epiphyseal cartilage was decreased in width in one animal (Table III, group C, W79).

The histological changes in the epiphyseal line shown in Figure 12 were both constant and marked in all the hypophysectomized rats of this group. The cells of the zone of proliferation were small, their nuclei pyknotic and they were reduced in number. The columns were regular in arrangement but much narrower than in the unoperated rats and thus there was a relative increase in the amount of matrix between them. The cells of the vesicular zone appeared shrunken, the lacunae small and there was a consequent reduction in the width of the zone. The erosion zone was inactive. In the diaphysis, the disappearance of the trabeculae was very striking. Their disappearance cannot be explained as a cessation of growth; there was an actual resorption of bone, but with no increase in the number of osteoclasts. The remaining trabeculae were large and well oriented. The marrow showed a tremendous increase in fat content at the expense of the myeloid elements.

Effect of Growth Hormone on Hypophysectomized Rats

The response of the hypophysectomized animal to growth hormone injections was very much greater than that of the unoperated rat. The cortical bone had a tendency toward increased thickness (Table III, compare groups C and D, column 1). The average width of the epiphyseal cartilage was even greater than in the unoperated injected rats (Table III, groups A and D, column 3); compared with the hypophysectomized controls (group C), the average increase in width was 53 units.

The histological differences between these animals and their operated controls were still more marked than were the differences in measurements (Fig. 13). The cells of the hyaline cartilage covering the epiphysis and their lacunae were larger, the nuclei were karyolytic, the cytoplasm vesicular and the cells as a whole less basophilic than in the hypophysectomized controls. The number of cells in the zone of proliferation of the epiphyseal cartilage increased tremendously with subsequent increase in length of the columns. The matrix became less homogeneous in character, taking the hematoxylin in streaks of blue. The cells of the vesicular zone and their containing envelopes of matrix were larger; the

nuclei of the cells were either karyolytic or entirely lacking. The erosion zone was extremely active and packed with cells, in marked contrast to the same zone in the hypophysectomized controls. The diaphysis was filled with numerous small trabeculae that were covered with osteoblasts, and osteogenesis as well as chondrogenesis was extremely active. The marrow recovered from the effects of hypophysectomy with a reduction in the number of fat cells and an increase in the myeloid components.

The rapidity and sensitivity of the response of the hypophysectomized rat to growth hormone injections was shown in the case of one animal (Table III, group D, GH84) that died because of alizarin red injections at 48 days of age after only 4 injections of the hormone. The histological changes in the tibia of this rat were comparable in all respects to the changes in the bones of animals injected over the 10-day period. However, the gain in body weight was only 5 gm. as compared with 20 and 24 gm. increases for the other two animals in the same group.

RAT TIBIA AT 88 DAYS

Effect of Growth Hormone on Unoperated Rats

The measurements of the various regions of the tibia are presented in Table IV. The cortical bone and articular cartilage showed no pronounced changes in width (compare groups A and B, columns 1 and 2). The average increase in width of the epiphyseal disk (column 3) was 7 units, again showing a very slight difference. There is no correlation in either this group or in that of animals 54 days old between the total body weight and the width of the epiphyseal line. The gross changes are shown in the survey pictures (Figs. 2 and 3).

The histological differences between the injected and the control animals of this group were more marked than in the preceding group (compare Figs. 6 and 7). In the unoperated animals of the group 54 days old, changes following injections were confined to the zone of erosion but in these animals there were also noticeable differences in the epiphyseal cartilage. The zone of proliferation was wider in the injected animals; the cells increased correspondingly both in number and size and were less basophilic. Toward the epiphyseal end of the columns the cells became so closely packed that the transverse matrix septa between them

were frequently lacking. The transition between the zone of proliferation and that of the vesicular cells was less abrupt, the karyolytic changes in the nuclei proceeding more gradually, especially in the peripheral areas of the disk. The lacunae of the vesicular cells were larger on the whole and the zone wider than in the controls, but the average number of cells was the same (See Figs. 2 and 9).

TABLE IV

Rats 88 Days Old: Summary of Measurements

Group	Rat No.	Gain in body weight, 10 days	Tibia, proximal portion		
			Cortical bone width*	Articular cartilage width*	Epiphyseal cartilage width†
		(gm.)			
A. Unoperated controls	BH09	27	16	11	33
	BH77	6	19	..	27
	BH94	20	25	19	34
Average		18	20	15	31
B. Unoperated injected	BH08	38	20	13	36
	W 72	43	20	13	41
	BH92	32	24	13	36
Average		38	21	13	38
C. Hypophysectomized	W 75	− 2	17	9	21
	BH05	− 7	17	11	14
	BH33	−12	23	13	16
	BH43	− 6	15	13	19
Average		− 7	18	12	18
D. Hypophysectomized injected	W 03	30	20	10	64
	W 73	42	18	7	74
	W 90	34	18	12	78
Average		35	19	10	72

* Measured with a calibrated ocular. One division is equivalent to 16.1 μ.
† One division equals 4.1 μ.

Effect of Hypophysectomy

There was a slight decrease in the average thickness of both the cortical bone and the articular cartilage in the animals of this group following the postoperative period of 25 days (Table IV, columns 1 and 2, compare groups A and C). The decrease in average width of the epiphyseal line (column 3) was 13 units, which was somewhat greater than the decrease in the younger animals. Figure 4 is a survey picture typical of the animals of this group. It shows the gross changes in the epiphyseal disk after hypophysectomy.

The histological changes in the disk are shown in Figure 8.

The picture of regression in the disk and bone atrophy is the same as previously described for the younger group, but it is more marked in degree.

Effect of Growth Hormone on Hypophysectomized Rats

There was a decrease in width of the articular cartilage not found in the rats 54 days old (Table IV, group D, column 2), but there was no change in the width of the cortical bone (column 1). The average increase in thickness of the epiphyseal cartilage over the operated controls was 54 units (column 3, compare groups C and D), an even greater increase than in the younger animals. A comparison of the survey pictures, Figs. 4 and 5, will show the magnitude of the response of these rats to the injections.

The histological changes in the epiphyseal cartilage, diaphysis and marrow shown in Figure 9 were identical in type with those already described for the hypophysectomized animals, 54 days old, injected with growth hormone, but the differences as compared with the control, Figure 8, were also more pronounced.

RAT TIBIA AT 150 DAYS

Effect of Growth Hormone on Unoperated Rats

The first group of animals 150 days old which was studied showed changes in the width and staining reaction of the epiphyseal disk that did not correspond with those found in the two groups previously described. Since it was felt that the difference was due to faulty technic in fixation and decalcification, a second series was prepared. The first animals in groups A, B, C and D, Table V (W97, B53, W88 and W58) belong to this series and were chosen as representative from a total of 15 rats. The following discussion will apply primarily to them. The remainder of the animals in Table V belong to the first group and must be considered separately as far as measurements are concerned.

The cortical bone (Table V, column 1) did not show any pronounced changes in thickness; there was a slight but inconclusive increase in the average width of the articular cartilage (column 2). The epiphyseal cartilage (column 3) was markedly increased in thickness in the first group, and increased to a less pronounced degree in the second (last three animals of groups A and B).

TABLE V

Rats 150 Days Old: Summary of Measurements

Group	Rat No.	Gain in body weight, 10 days	Tibia, proximal portion		
			Cortical width* bone	Articular cartilage width*	Epiphyseal cartilage width†
		(gm.)			
A. Unoperated controls	W97	6	25	11	40
	W07	0	24	..	18
	B15	6	24	..	20
	W23	2	25	10	18
B. Unoperated injected	B53	42	24	14	55
	W09	23	21	12	25
	W24	20	20	..	25
	B44	18	22	..	28
C. Hypophysectomized controls	W88	4	22	14	27
	B14	−30	23	..	12
	B27	−16	18	13	13
	B42	−16	22	..	15
D. Hypophysectomized injected	W58	10	20	8	56
	G10	23	..	14	50
	B16	−14	22	..	28
	BH45	16	25	..	27

* Measured with a calibrated ocular. One division is equivalent to 16.1 μ.
† One division equals 4.1 μ.

The histologic changes were the same in both groups. There were no essential differences in the cortical bone and articular cartilage between control and treated animals. The changes in the epiphyseal cartilage are shown in Figure 15. It was hypertrophied in all zones and much more regular in width. The cells in the zone of proliferation were larger, their nuclear detail was clearer and frequently several cells occupied a single lacuna—evidence of the rapidity of proliferation. The lacunae of the zone of vesicular cells were also enlarged, the matrix between them less abundant and less basophilic than in the controls. The nuclei of the cells were large and possessed two distinct nucleoli; their cytoplasm was abundant and "lacy" in character. Activity along the zone of erosion was confined to a narrow band which was more or less clearly set off from the rest of the medullary cavity. This line of activity was evident in these animals because of the relative inactivity of the bone prior to the period of injections; on the other hand, in younger animals such a band could not be clearly discerned because of the activity previous to the injections. As in

the groups of animals already described, there was a definite stimulation of the myeloid elements of the marrow at the expense of the fat cells.

Effect of Hypophysectomy

The first animals in groups C and D, Table V (W88 and W58) had been hypophysectomized for 25 days, the remaining 6 for a period of only 10 days; hence the difference in the loss of weight of the controls. There was a tendency toward a decrease in the width of the cortical bone and an increase of the articular cartilage. The measurements of the epiphyseal cartilage tended to lose their significance since the disk was very irregular in width.

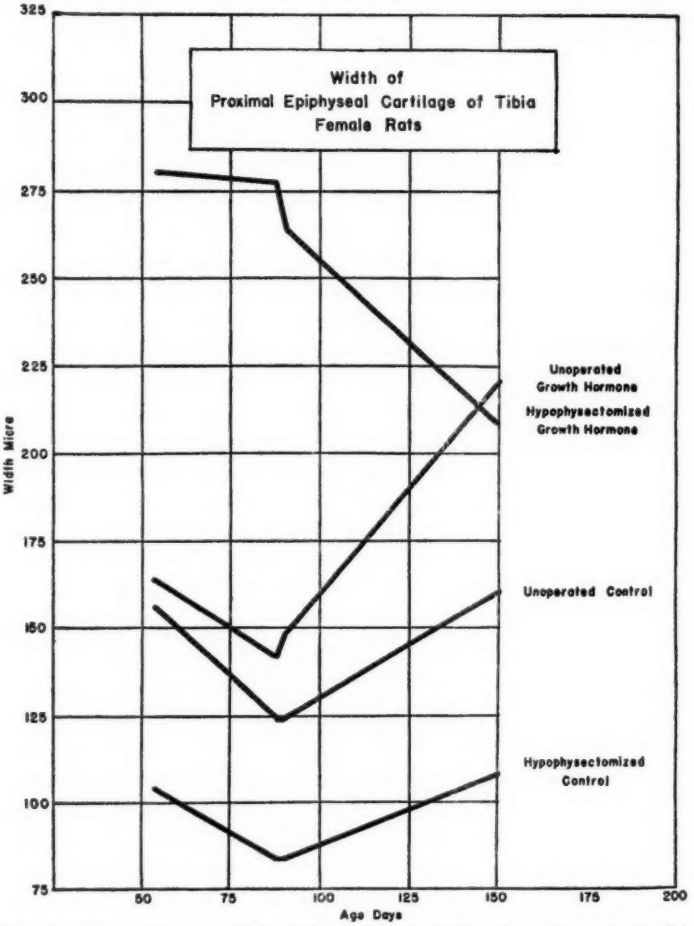
The epiphyseal cartilage was distinctly outlined on *both* sides by a layer of bone. The histological changes in this region are shown in Figure 16. The matrix in the zone of proliferation was relatively abundant because of the reduction in both the size and the number of cells. The cells themselves were extremely basophilic, the nuclei so much so that their detail could not be made out. The vesicular zone was very narrow and here even the matrix was basophilic. The cells of this zone were almost as small and basophilic as the cells of the preceding zone and in places there appeared to be transitions of these cells into osteocytes. The zone of erosion was lacking, and instead bone had been deposited in this region. In the diaphysis, the trabeculae were reduced in number, although the atrophy was not so marked as in the animals 88 days old.

Effect of Growth Hormone on Hypophysectomized Rats

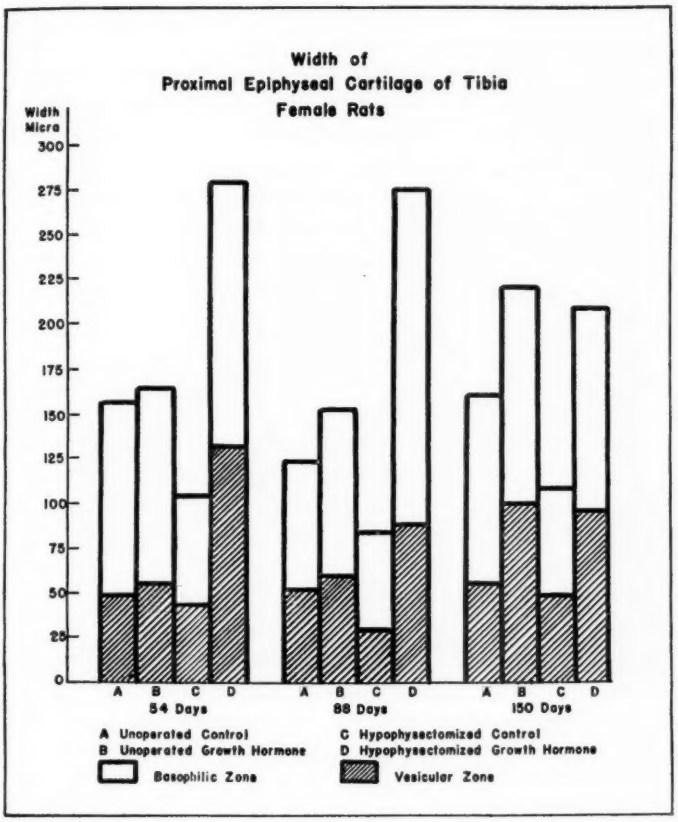
The measurements of the cortical bone and articular cartilage did not show any definite trends (Table V, group D, columns 1 and 2). The increase in width of the epiphyseal cartilage (column 3) was marked and could readily be seen in the survey picture, but it was nowhere near so great as in the rats 54 and 88 days old.

Histologically, the changes were essentially the same as in the injected, unoperated animals of the same age group. Previous to the injections, bone had been deposited along the diaphyseal side of the epiphyseal cartilage. This layer clearly delimited the activity, following injections, from the rest of the diaphysis, although it did not prevent the cartilage from responding. A clear

line such as this was not observed in the younger animals. It probably corresponds to the "line of arrested growth" common in many pathological processes as described by Harris.[10] The response of the epiphyseal cartilage and the increase in activity along the zone of erosion are shown in Figure 16. A final point of interest in this group was that one animal (Table V, group D, B16) continued to lose weight during the period of injection and yet showed pronounced stimulation of endochondral ossification.



TEXT-FIGURE 3. Average width of the proximal epiphyseal cartilage of the tibia plotted against the age (54, 88, 90 and 150 days) at autopsy.



TEXT-FIGURE 4. Average width of the proximal epiphyseal cartilage of the tibia at the ages indicated.

DISCUSSION

Text-Figures 3 and 4 summarize graphically the data on the widths of the epiphyseal cartilage (Tables III to V).

The greatest difference in width between normal control and injected animals came at 150 days of age. This may indicate an increase in the sensitivity of the response with advancing age, an observation to be expected since skeletal growth is already so active in young rats that it would be difficult to stimulate it further. Following hypophysectomy the reduction in width of the epi-

physeal cartilage was proportional in all three age groups, indicating, together with the histological changes, an active process rather than a passive cessation of growth. If the latter were the case one would not expect to find such distinct changes in skeletally mature animals. The response of hypophysectomized animals to injections was much more pronounced than in the unoperated rats. This may indicate that some fraction normally present in the pituitary antagonizes the action of growth hormone. Finally, the response of the epiphyseal cartilage in the operated rats, in contrast to that in the unoperated animals, was less marked at 150 days of age than at 50, which may be interpreted as a reduction in sensitivity with increasing age.

These observations differ in several respects from those of Freud, Levie and Kroon,[1] who reported that growth hormone primarily affects the proliferating cartilage. The fraction used in this laboratory also caused marked stimulation of endochondral ossification and coincident connective tissue metaplasia. In addition, definite stimulation of the myeloid components of the marrow followed injections. Freud, Levie and Kroon also reported that the effects of hypophysectomy were localized in the epiphyseal cartilage and that epiphyseal closure followed the operation. However, Figure 4 shows, in addition to changes in the disk, definite atrophy of the trabeculae and marked increase in the fat content of the marrow.* The deposition of bone along the epiphyseal cartilage described by Freud, Levie and Kroon depends to some extent on the age of the animal, but even in rats 150 days old after a postoperative period of 2 weeks this did not prevent the response of the disk to injections. Following a postoperative period of 3 to 4 weeks there was still no definite evidence of "irreversible" epiphyseal closure. It may be concluded that the action of the anterior pituitary fraction used in this laboratory and also the effect of hypophysectomy are not confined to the epiphyseal disk but influence the whole process of endochondral ossification and coincident connective tissue metaplasia in the tibia. The effect of this fraction on normal periosteal ossification is less marked, which may explain why

* It has been reported by Gaebler [11] and Evans, Luck, Pencharz and Stoner [12] that hypophysectomy raises the respiratory quotient due to decreased utilization of fats. The reverse takes place following growth hormone injections. This may be the biochemical explanation for the histological findings in the marrow.

Handelsman and Gordon [4] were unable to get a more sensitive reaction in the skull and mandible of the rat.

Silberberg's [5] observation that epiphyseal closure soon follows the onset of injections may be explained on the basis of the fraction used. An acid extract of the anterior pituitary would probably be rich in gonadotrophic fractions which may cause calcification of the epiphyseal line.

The data previously presented (Tables III to V) tend to confirm the observations of Freud, Levie and Kroon [1] that the response of the epiphyseal cartilage of the tibia to growth hormone is much more constant than the gain in body weight. An extreme illustration of this is one of the hypophysectomized rats, aged 150 days, which continued to lose weight during growth hormone treatment (Table V, B16) but which showed changes in the tibia comparable with the other animals in the same group. Furthermore, the response of the tibia was much more rapid and sensitive than the increase in body weight, as previously pointed out for an hypophysectomized rat 54 days old (Table III, GH84) that died after only 4 injections.

Conclusions

1. Early in the life of a normal female rat (between 25 and 50 days of age) there is an equilibrium established between the formation of cartilage and bone in endochondral ossification, an equilibrium which, in the proximal end of the tibia, is maintained until very late in the life of the animal.

2. Injections of growth hormone over a short period (10 days) in normal animals are followed by definite stimulation of endochondral ossification with little disturbance of this equilibrium, except in rats 150 days old in which there is hypertrophy of the epiphyseal cartilage. Injections are followed also by a decrease in the fat content of the marrow.

3. Hypophysectomy is followed after 25 days not only by (a) disturbance of this equilibrium with a reduction in the width of the epiphyseal cartilage; but also by (b) resorption of the diaphyseal trabeculae; (c) an increase in the fat content of the marrow; and (d) in animals 150 days old, a deposition of bone along the diaphyseal side of the epiphyseal cartilage.

4. Injections of growth hormone over a similar period in

hypophysectomized animals (15 days postoperative + 10 daily injections = 25 days) are followed by (a) definite disruption of the equilibrium with increased cartilage formation and a return to the "youthful" type of epiphyseal line, most pronounced in young animals (54 to 88 days of age); (b) increased activity in the diaphysis with the formation of trabeculae and the deposition of bone along them, and (c) stimulation of the myeloid elements of the marrow with a reduction in the number of fat cells.

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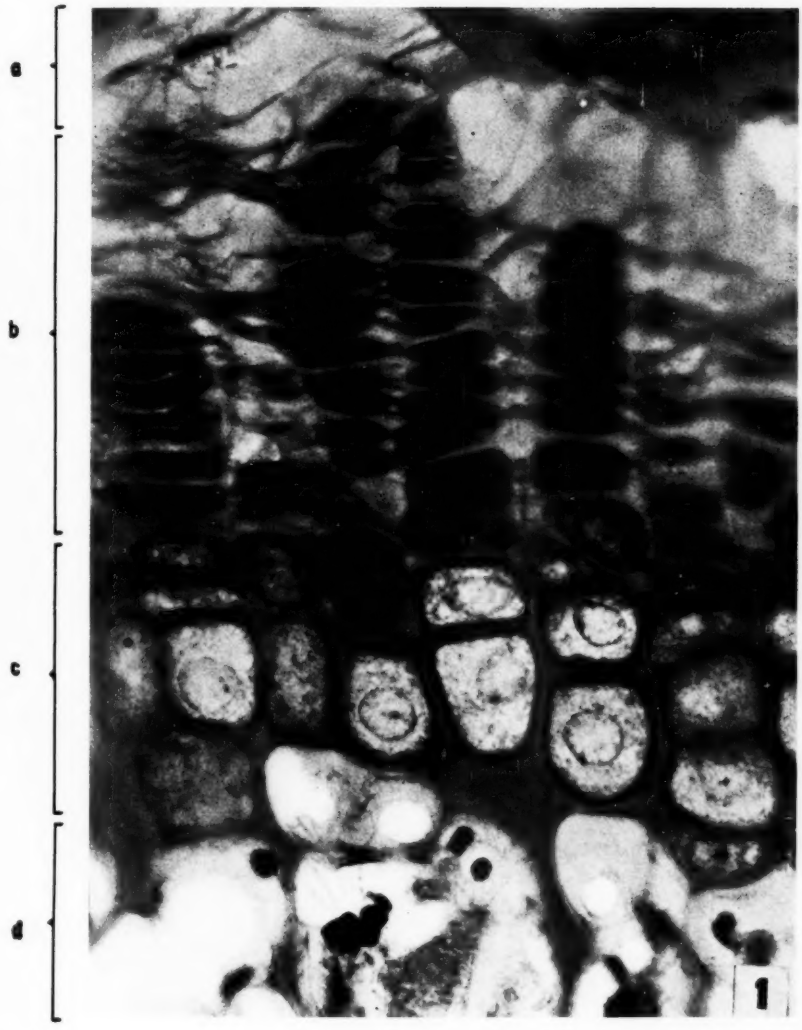
DESCRIPTION OF PLATES

PLATE 97

FIG. 1. Epiphyseal disk of a normal adult rat. (a) Remnant of embryonic hyaline cartilage; (b) zone of basophilic cells; (c) zone of vesicular cells; (d) zone of erosion.



Ray, Evans and Becks Effect of Pituitary Growth Hormone

PLATE 98

FIGS. 2 to 5. Survey photomicrographs of the proximal epiphysis of the tibias of female rats, 88 days of age at autopsy.

FIG. 2. Control animal (BH09).

FIG. 3. Experimental animal (W72) receiving ten daily injections of growth hormone prior to autopsy.

FIG. 4. Hypophysectomized control animal (W75), 25 days postoperative.

FIG. 5. Hypophysectomized animal (W73), 25 days postoperative, given ten daily injections of growth hormone prior to autopsy.

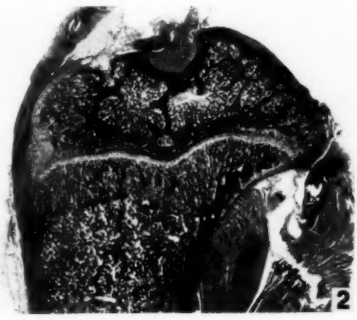
FIGS. 6 to 9. Changes in the central area of the epiphyseal disk in female rats 88 days of age at autopsy.

FIG. 6. Control animal (BH09).

FIG. 7. Experimental animal (W72) receiving ten daily injections of growth hormone prior to autopsy.

FIG. 8. Hypophysectomized control (W75), 25 days postoperative.

FIG. 9. Hypophysectomized animal (W73), 25 days postoperative, given ten daily injections of growth hormone prior to autopsy.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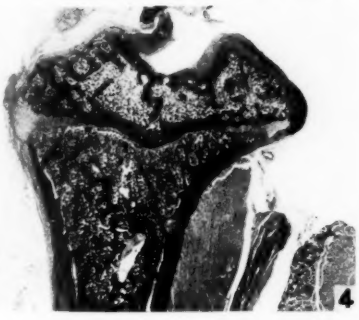
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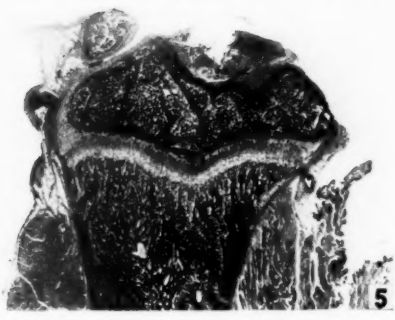
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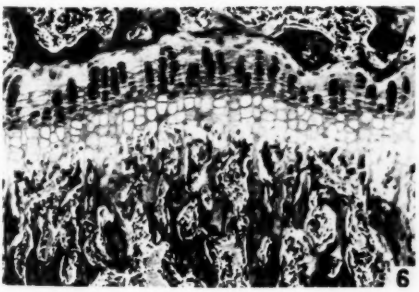
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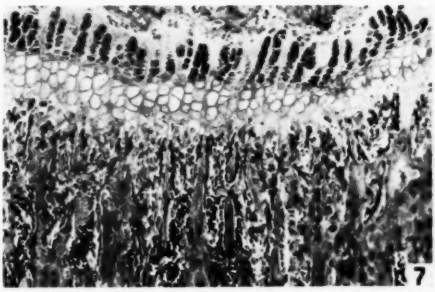
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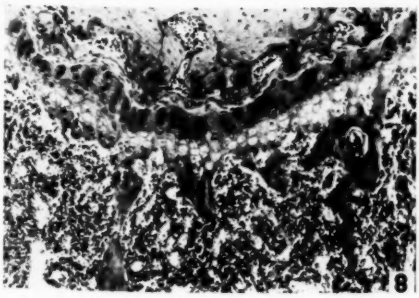
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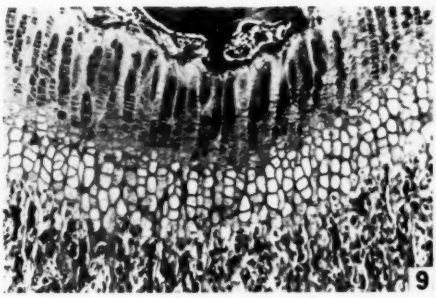
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PLATE 99

FIGS. 10 to 13. Changes in the central area of the epiphyseal disk in female rats, 54 days of age at autopsy.

FIG. 10. Control animal (W82).

FIG. 11. Experimental animal (W53) receiving ten daily injections of growth hormone prior to autopsy.

FIG. 12. Hypophysectomized control (G32), 25 days postoperative.

FIG. 13. Hypophysectomized animal (G33), 25 days postoperative, given ten daily injections of growth hormone prior to autopsy.

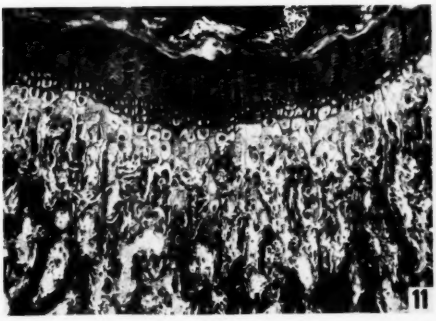
FIGS. 14 to 17. Changes in the central area of the epiphyseal disk in female rats, 150 days of age at autopsy.

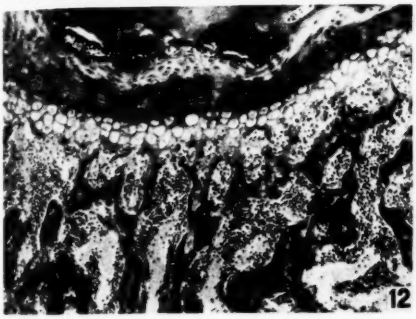
FIG. 14. Control animal (W97).

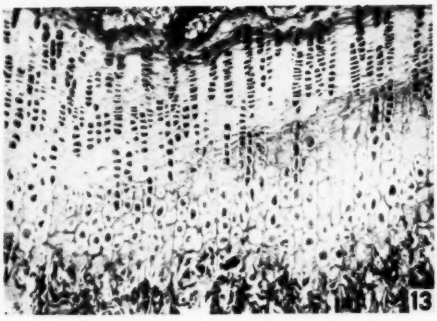
FIG. 15. Experimental animal (B53) receiving ten daily injections of growth hormone prior to autopsy.

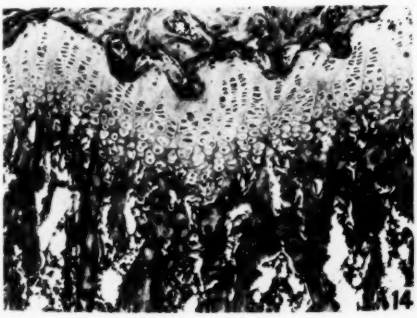
FIG. 16. Hypophysectomized control (W88), 25 days postoperative.

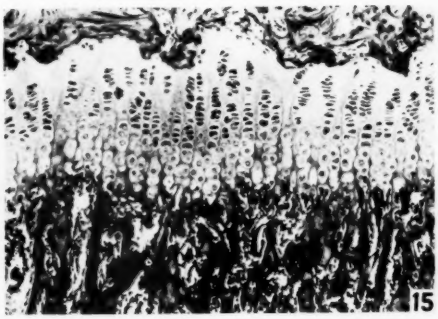
FIG. 17. Hypophysectomized animal (W58), 25 days postoperative, given ten daily injections of growth hormone prior to autopsy.

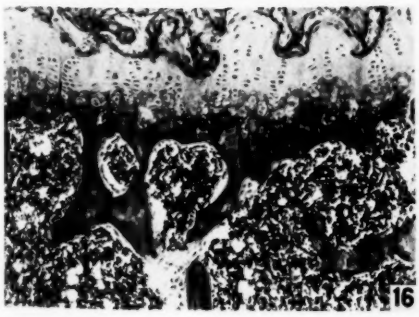


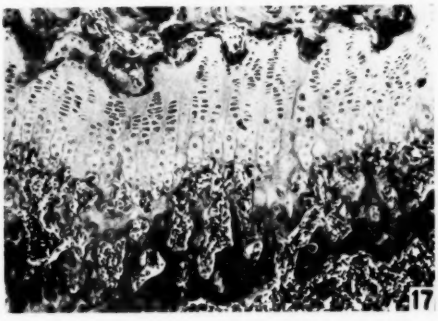












Ray, Evans and Becks Effect of Pituitary Growth Hormone

CHANGES IN THE INCISOR TEETH OF ALBINO RATS WITH VITAMIN A DEFICIENCY AND THE EFFECTS OF REPLACEMENT THERAPY *

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"The pathology of vitamin A deficiency indicates that the seat of the physiologic disturbances is in the epithelial cells. Chemical rôles are suppressed but proliferative powers are not inhibited; neither are the potentialities of cells lost, as is shown by the return to normal physiologic function when vitamin A is restored to the animal."—Wolbach.

The classic work of Wolbach and Howe [1, 2] on the dental changes in vitamin A deficiency stimulated this investigation. The purposes of this study were to repeat some of their work; to study the effects of chronic vitamin A deficiency; to measure the rate of appositional growth of dentin in vitamin A deficiency; and to interpret the findings in the light of the more recent knowledge of the histophysiology of the rat incisor.

REVIEW OF LITERATURE

The dental findings of Wolbach and Howe [2] may be summarized as follows: (1) The initial effect upon the incisor teeth of rats consisted of an atrophy of the enamel organ which began in the anterior portion and finally extended to the whole length of the tooth. (2) Atrophy and depolarization of the odontoblasts followed the changes in the enamel organ. The odontoblasts showed more severe alterations on the lingual side where the dentin was thin, folded, or absent. The odontoblasts survived longer on the labial side where the dentin was excessively wide. Osteoid tissue and epithelial cells derived from the enamel organ were found in the pulp. (3) Effects of replacement therapy with butter fat were noted within 7 days. Repair began in the region of Hertwig's epithelial sheath and was manifest by a recovery of the enamel organ and resumption of the normal morphology and function of the odontoblasts. Tubular predentin was now

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deposited. The epithelial cells in the pulp were surrounded by dentin, which in turn was surrounded by odontoblasts. Excessive local formation of atypical dentin increased with the recovery period. Morphological recovery was complete in 19 days.

Wolbach and Howe [2] used a diet that was deficient in vitamins A, C, D and E, but indicated that the lack of vitamins C, D and E was insignificant in respect to complicating the vitamin A-deficiency picture in the rat. They found that the addition of vitamin A alone was sufficient to bring about histological recovery. In a later report Wolbach [3] gave the following summary on the teeth in vitamin A deficiency: "The continuously growing incisor teeth of rodents—rats and guinea-pigs—are profoundly affected owing first to atrophy and metaplasia of the enamel forming organ and subsequently to atrophy and cessation of or irregular functioning of odontoblasts. Enamel formation is suppressed, and striking deformities of the dentin result."

Boyle [4] described, in the tooth germ of a human infant with vitamin A deficiency, changes in the enamel organ which were similar to those found by Wolbach and Howe [2] in the rat incisor. Mellanby and King [5] found hyperplasia of the gingivae and periodontal disease in dogs, rabbits and rats placed on a diet deficient in vitamin A. King [6] confirmed previous findings in dogs, and in addition reported retarded eruption and malposition of the teeth; malformation of the roots associated with hypoplastic changes in Hertwig's epithelial sheath; ill-defined laminae durae; changes in alveolar bone and a tendency to apical hypercementosis. King [7] also studied the effects of vitamin A deficiency in the rat and emphasized the disturbance in the calcification of dentin in spite of the fact that the animals were given ample amounts of vitamin D, calcium and phosphorus. Smith and Lantz[8] reported a loss of normal pigment and a dull white, opaque appearance of the incisors of rats placed on a vitamin A-deficiency ration. The teeth were short and blunt. Eruption was markedly retarded. Fridericia and Gudjonsson [9] also reported progressive retardation in the eruption of the incisors of rats in vitamin A deficiency. Orten, Burn and Smith [10] studied the effects of prolonged incomplete vitamin A deficiency in the incisor of the white rat. They reported tumor growths (odontomata) which arose from the pulp and proliferated in some cases to the point

of replacement of the alveolar bone. Pohto [11] found atrophic changes in the odontoblasts that were similar to those found by Wolbach and Howe.[2] He emphasized the peglike dentin projections and the prominent foldings. The latter were present in the labial as well as the lingual dentin when the vitamin A deficiency was prolonged.

More recently Mellanby [12] reported on the changes in the incisors and molars of young rats whose mothers received a diet deficient in vitamin A for 5 to 7 months. The incisors showed degeneration of the enamel organ and of the ameloblasts and reduction of blood supply. Enamel was lacking in some areas. The odontoblasts degenerated on the lingual side. The dentin was poorly calcified and distorted in outline. The molars also showed defective enamel formation and poor calcification of dentin. The pulp contained ossifying areas. Mellanby emphasized the disturbance in the organizing action of the enamel organ.

For additional references the reader is referred to Wolbach and Howe [2] and Pohto.[11]

Material and Methods

This study is based on 199 rats which were placed, at weaning, on a diet deficient in vitamin A for a period of 9 to 81 days. The animals were weaned at 21 days of age.

The diet consisted of:

Cornstarch	66.5 per cent
Casein (Vitamin A free)	18.0 per cent
Brewer's yeast	10.0 per cent
Osborne and Mendel's salt mixture	4.0 per cent
Sodium chloride	1.0 per cent
Irradiated cholesterol	0.5 per cent

The animals were weighed weekly. Vitamin A reserve was considered to be depleted when the animals became stationary in weight and showed early signs of xerophthalmia (21 to 26 days). Twelve animals of the same colony, placed on the normal stock diet, were used as controls. The experimental animals were grouped as follows:

Group I consisted of 70 rats on vitamin A-deficient diet without replacement therapy (Table I). These animals were placed on the deficient diet for a period of 26 to 56 days following weaning. They did not survive longer than 56 days.

TABLE I

Group I. Seventy White Rats Placed on a Vitamin A-Deficient Diet Arranged According to Duration of Survival Period

Subgroups	Experimental history				Findings		
	Number of animals	Age at beginning of experiment	Age at death	Duration of experiment	Gross	Dental	
						Radiographic	Histopathologic
		(*days*)	(*days*)	(*days*)			
A	12	21	45–47	24–26	Sensitive to light; cessation in increase in weight	—	− (+)
B	17	21	55–59	34–38	Xerophthalmia; mild cessation in increase in weight	+	++
C	32	21	65–69	44–48	Severe xerophthalmia; loss in weight	++	+++
D	9	21	73	48–56	Very severe ophthalmia; loss in weight and near death	++++	++++

Number of plus (+) signs indicates degree of severity of changes.

Group II consisted of 34 rats on a vitamin A-deficient diet with replacement therapy consisting of additions of definite amounts of alfalfa or cod liver oil. Twelve animals of this group were fed a vitamin A-deficient diet for 25 days after weaning. Suboptimal rations of alfalfa were then added to the basal diet for 8 weeks. These animals survived longer than those of group I and were sacrificed at the age of 102 days (Table II). Four animals were placed on 1 per cent alfalfa-leaf meal concurrently with the basal vitamin A-deficient diet for 56 days. The remaining animals were given total replacement therapy with cod liver oil after varying periods of vitamin A deficiency.

Group III. Ninety-five albino rats were placed on a complete vitamin A-deficient diet at 21 days of age and given intraperitoneal injections of 0.5 cc. of a 2 per cent solution of alizarin red S at intervals of 4 to 11 days in order to find the rate of apposition of the dentin in the incisor and of the dentin, cementum and

TABLE II

Group II. Thirty-four White Rats Placed on a Vitamin A-Deficient Diet Plus a Suboptimal or Total Replacement Diet Arranged According to Duration of Survival

Sub-groups	Number of animals	Age at beginning of experiment	Length of experiment prior to giving of replacement	Duration of complete experiment	Duration of replacement diet	Type of replacement	Age at death	Findings		
								Gross	Radiographic	Histopathologic
		(*days*)	(*days*)	(*days*)	(*days*)		(*days*)			
A	4	21	0	56	56	Alfalfa 1%	77	Normal	....	....
B	12	21	25	81	56	Suboptimal alfalfa	102	Xerophthalmia and loss in weight	++++	++++ No signs of repair
C	12	21	29	44–54	15–25	Total replacement with cod liver oil	65–75	Complete recovery from xerophthalmia and weight loss	+	+ Active reparative processes in proximal half of incisor
D	6	21	44	54–64	10–20	Total replacement with cod liver oil	75–85	Mild recovery from xerophthalmia and no gain in weight	+++	+++ Reparative process beginning

Number of plus (+) signs indicates degree of severity of changes.

TABLE III

Part of Group III. Sixty Albino Rats on a Vitamin A-Deficient Diet and the Daily Rate of Dentin Apposition in Their Incisor Teeth

Sub-groups	Number of animals*	Age at which alizarin red S was injected	Replacement therapy and ages at which started	Age at death	Average rate of apposition per 24 hours				Approximate ratio between daily rates at midlingual and at midlabial regions
					Mid-labial	Disto-lingual	Mesio-lingual	Mid-lingual	
		(*days*)		(*days*)	(μ)	(μ)	(μ)	(μ)	
1	45	30, 40	None	45	16.14	14.38	13.76	13.42	3:4
		43, 54	None	73	16.79	13.11	12.48	10.86	2:3
		47, 57	None	77	17.91	12.85	12.13	9.57	1:2
		50, 60	None	65	18.68	11.14	10.75	7.84	1:2
		64, 72	None	77	19.63	11.29	9.01	6.43	1:3
2	8	47, 51	Insufficient suboptimum vitamin A replacement as blue gramma grass at 47 days	52	18.32	12.47	11.83	8.91	1:2
3	7	50, 60	Full replacement at 50 days	75	13.12	15.78	15.76	15.81	1:1
		60, 70	" " " " "	75	15.97	15.83	15.80	15.91	1:1

* All rats were placed on vitamin A-deficient diet at 21 days of age (weaning).

TABLE IV

Part of Group III. Thirty-five Albino Rats on a Vitamin A-Deficient Diet and the Effects of Cod-Liver-Oil Replacement on the Rates of Dentin Growth in Their Incisor Teeth

Sub-groups	Number of animals*	Daily units of cod liver oil	Age at beginning of replacement	Age at which alizarin was injected		Age at death	Daily rates of dentin growth		Ratio of midlingual to midlabial
				1st injection	2nd injection		Lingual	Labial	
			(*days*)	(*days*)	(*days*)	(*days*)	(μ)	(μ)	
1	5	1	42–48	50	60	65	8.35 ± .74	15.93 ± .08	1:2
2	5	2	42–48	50	60	65	12.54 ± .40	16.66 ± .33	3:4
3	5	3	42–48	50	60	65	12.40 ± .21	16.76 ± .10	3:4
4	5	4	42–48	50	60	65	12.62 ± .13	16.10 ± .16	3:4
5	5	5	42–48	50	60	65	15.52 ± .24	15.91 ± .17	1:1
6	4	1	42	42	52	56	8.46 ± .37	17.80 ± .09	1:2
7	3	2	42	42	52	56	12.11 ± .16	17.08 ± .29	3:4
8	3	5	42	42	52	56	15.75 ± .22	15.95 ± .36	1:1

* All animals were placed on a vitamin A-deficient diet at 21 days of age and carried on the deficient diet until outward symptoms (weight gain and xerophthalmia) appeared.

alveolar bone in the molar. The animals were sacrificed from 5 to 30 days following the initial injections. Fifty animals of this group were given various levels of replacement therapy (Tables III and IV).

Radiographic and Histologic Methods

The dietary experiments and the administration of alizarin red S were carried out in the chemical laboratory at Tucson, Arizona (M. C. S.), where the gross conditions of the living animals were also observed and recorded. After death the animals were decapitated and the heads were fixed in a 4 per cent aqueous solution of formaldehyde and sent to the histologic laboratory of the College of Dentistry, University of Illinois, where the radiographic and histologic studies were carried out.

The heads were split in halves by a midsagittal cut between the left and right incisors. Each half was radiographed by exposure on a dental occlusal film for 5 seconds, 5½ in. from the aperture of the tube casing, without the cone attached. A standard dental X-ray machine was used.

The jaws of groups I and II were washed and then de-

calcified in 5 per cent nitric acid for 24 hours. After dehydration and embedding in celloidin, serial midsagittal (longitudinal) and transverse sections of the incisors were stained with hematoxylin and eosin and mounted. Our studies were based mainly on longitudinal sections which facilitate the ready tracing of events from the basal to the anterior level.

The teeth of the animals that were given injections of alizarin red S were studied in ground as well as in decalcified sections. Transverse sections of upper and lower incisors were prepared by grinding on a medium and then a fine carborundum stone mounted on a dental lathe. Longitudinal ground sections of the uppers were also prepared. The lower incisors, because of the marked twist in their anteroposterior axes, were cut in half at the level of the first molar, and each half was then ground.

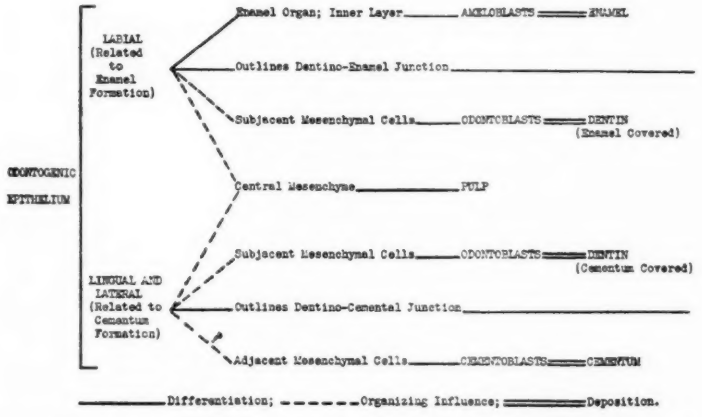
Measurements were made with a filar micrometer eyepiece, standardized to a stage micrometer. By measuring the distance between any two injection effects and dividing this by the time interval the daily rates of apposition were obtained.[13] The figures on the appositional rates were subjected to statistical evaluation. Transverse and longitudinal ground sections were also prepared of the incisors of representative animals of groups I and II.

Histophysiology of the Rat Incisor

Before presenting the findings in the experimental animals, we shall consider briefly those histophysiologic aspects of the incisor of the rat that have a particular bearing on the experimental changes analyzed in this report and that have become clarified during the progress of this study. The development of the incisor of the rat consists of four main stages: growth, calcification, eruption and attrition. For the purposes of this study we are primarily interested in the growth process, which passes successively through the proliferative, differentiative and appositional phases.

Odontogenic Epithelium. The rat incisor develops primarily from an elliptical epithelial base which is situated at the proximal end of the tooth and which proliferates throughout the life of the animal. Because of its function, this base may be called the odontogenic epithelium.[14] It establishes the dentino-enamel and dentino-cemental junctions and thus the size and shape of the tooth. The labial third of the epithelial base overlaps a portion of the lateral surfaces (Fig. 22) and resembles in structure and in function the enamel organ of the human tooth. It establishes the dentino-enamel junction. Its inner layer, the inner enamel

epithelium, differentiates into ameloblasts and, in addition, regulates and activates the subjacent mesenchymal cells facing the ameloblasts to differentiate into odontoblasts. The remaining two thirds of the odontogenic epithelium resembles in structure Hertwig's epithelial sheath and outlines the lingual and the larger portion of the lateral surfaces. It establishes the dentino-cemental junction, activates the subjacent cells of the pulp to differentiate into odontoblasts, and possibly also activates the adjacent cells of the dental follicle to differentiate into cementoblasts (Text-Fig. 1). Thus there is a striking morphologic and functional difference between the labial and lingual aspects of the odontogenic epithelium. This difference is foreshadowed even in the newborn before apposition has begun.[15] For convenience we shall therefore refer to the labial odontogenic epithelium and the lingual odontogenic epithelium. Similarly, we shall distinguish between the labial or enamel-covered dentin and the latero-lingual or cementum-covered dentin, and between the corresponding labial, lateral and lingual odontoblasts.



TEXT-FIGURE 1. The histodifferentiation and organization of odontogenic epithelium.

Ameloblasts and Enamel. The ameloblasts recede peripherally from the dentino-enamel junction up to the point of maximum width of enamel, when they cease their activity in enamel apposition. The ameloblasts retain their columnar shape for approxi-

mately 50 days, when they become reduced and atrophied. In the adult rat (100 days or older) this state is usually reached in the anterior third of the incisor. In the young rat of 25 days of age, on the other hand, the ameloblasts maintain in the upper incisor their columnar shape even up to the gingival crest, because they require only 25 days to reach this level.

Odontoblasts and Dentin. As soon as the peripheral cells of the dental papilla differentiate into odontoblasts, they help form the dentin matrix, recede centrally, and migrate with the eruption of the tooth toward the distal end.

The pulpal recession of a given odontoblast is proportional to the amount of dentin that is laid down. The daily rate of dentin deposition is 16 μ in 24 hours.[13] The forward movement is in proportion to the rate of eruption which is about 2 mm. a week in the upper incisor and 2.8 mm. a week in the lower incisor.

In the incisor of a rat

TABLE V

Maximum Width of Dentin at Various Ages in the Incisor of the Normal Rat and that of the Vitamin A-Deficient Rat

Age	Normal controls				Vitamin A-deficient rats				Age‡
	Maximum width at various anatomic locations*				Maximum width at various anatomic locations†				
	Labial	Mid-lingual	Mesio-lingual	Disto-lingual	Disto-lingual	Mesio-lingual	Mid-lingual	Labial	
(days)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	*(days)*
58	504	489	483	497	475	416	309	511	54
60	528	505	507	508	463	421	331	531	57
70	656	587	594	610	492	430	342	658	65
77	727	634	643	678	408	374	350	744	77
100	910	849	875	887	593	541	357	1363	102

* Measurements made from transverse sections at incisal edge.
† Maximum width is attained at a more proximal level.
‡ All vitamin A deficiencies were started on the 21st day of life. Complete depletion of reserve occurs approximately 26 days later.

about 100 days of age the maximum thickness of the labial dentin is found at the incisal end and is approximately 900 μ (Table V). This is associated with the fact that the life span of the odontoblasts is approximately 55 days and the daily rate of apposition is 16 μ. Normally the width of the dentin, though slightly less in the cementum-covered portion, is similar throughout the circumference of the cross section of the incisor so that the ratio between the widths at the midlabial and midlingual levels is essentially 1:1.

Very little is known regarding the mechanism of the formation and calcification of enamel and dentin. There is, however, clear evidence of an intimate interrelationship between these processes. Thus normally the following orderly sequence is observed in the development of the tooth: Proliferation of the ameloblasts, differentiation of the ameloblasts, differentiation of the odontoblasts, formation of dentin matrix, formation of enamel matrix, calcification of dentin, calcification of enamel. An arrest of one step means the omission or disturbance of the subsequent processes (Text-Fig. 1).

For a detailed discussion of other phases of the normal histophysiology of the incisor of the rat the reader is referred to Schour and Steadman,[14] Addison and Appleton,[15] and Schour and Massler.[16]

Results

General gross findings

Group I. The vitamin A body reserves were considered to be exhausted between the 24th and 26th day following the beginning of the diet. This exhaustion was indicated by cessation in gain in body weight and by slight sensitivity to light. Xerophthalmia became evident in a mild form in 34 days. Its severity gradually increased in those groups which survived 45 days or more (Table I). The gross changes in the animals of this series were similar to those reported by Smith and Lantz.[8]

Group II. Those animals which received large doses of alfalfa in their diet together with their basal vitamin A-deficient ration showed no outward changes.

Group III. The gross effects in group III were similar to those of group I. However, on dissection, the bones of the skull

and jaws showed a reddish tint as a result of the alizarin injections.

The normal control rats showed no abnormal outward changes.

RADIOGRAPHIC FINDINGS

The normal controls showed no abnormal radiographic changes (Figs. 1 and 5).

Group I. The rats of subgroup A (Table I), which survived only 26 days, showed no abnormal radiographic changes in the incisors. The rats of subgroups B, C and D showed radiographic disturbances which progressed with the increase in survival length (Figs. 1–8).

The roentgenogram of the incisor of a normal rat presents a curved and partially hollow cylinder. The shadow is practically solid in the distal third and beginning with the middle third splits into nearly equal convex (labial) and concave (lingual) borders which taper in the proximal direction and surround the centrally located pulp (Fig. 1). In contrast, the shadow of the incisor of a rat subjected to vitamin A deficiency shows a striking distortion and gives the appearance of a sickle (Figs. 4 and 8). The convex border which represents the enamel-covered dentin is excessively wide, and at its proximal end makes a sharp bend toward the pulp. The latter is displaced toward the lingual border. The concave border, which represents the lingual dentin, is often not seen in the proximal third of the incisor and appears as a fine line only in the middle third (Figs. 4 and 8). The form of the tooth is thus distorted in a characteristic manner so that it is possible to diagnose the condition of vitamin A deficiency by an examination of the roentgenogram of the incisor of the rat (Figs. 1 to 8).

A more detailed analysis shows the following characteristic changes which permit a diagnosis of vitamin A deficiency on the basis of the roentgenogram (Figs. 2 to 4, 6 to 8):

1. The labial surface is often irregular and is foreshortened at its proximal end. Here it deflects abruptly from the normal curvature of the tooth toward the pulp.
2. The labial alveolar periosteum is widened to about three times the normal.
3. The alveolar bone at the base of the tooth tends to be more distinct and thickened.

4. Enamel hypoplasia is common. In the animals with a longer survival time the proximal end of the enamel is slightly buckled and often presents a picture which simulates that of a vesicle or hollow kernel (Figs. 2, 4, 7 and 8). This differs from the usual picture of hypoplasia in that the crest of this vesicle may extend beyond the height of the enamel surface. Histologic analysis showed that these vesicles were circumscribed areas in which enamel and dentin were defective or absent and the pulp communicated with the labial alveolar periosteum through a perforation by connective tissue (Figs. 10 and 17).
5. The labial dentin is increasingly and abnormally thickened toward the distal end.
6. The pulp is thus displaced lingually.
7. The lingual dentin is very thin in the middle third and often cannot be seen in the proximal third, indicating its absence or its lack of calcification.
8. The width of the periodontal membrane is irregular and narrow, particularly at the midregion.
9. In the upper incisor the extra-alveolar portion is longer than normal and the intra-alveolar portion is shorter than normal (Figs. 1, 4 and 8). The total length of the upper incisor is, however, not longer than normal. It appears as if a portion of the tooth which is normally intra-alveolar in position has become extruded.
10. In the lower incisor the extra-alveolar portion is shorter than normal. The intra-alveolar portion usually extends proximally only as far as the mesial level of the third molar, while normally it extends beyond the third molar toward the sigmoid notch of the ramus of the mandible.
11. The incisal relationship and attrition are abnormal. The incisal bevels show a less acute angle than normal.

Group II. In the group which received minute doses of alfalfa during the last 8 weeks of life, in addition to their vitamin A-deficient basal diet, the changes were more prominent but closely paralleled the findings in subgroup D of group I (Table II, Figs. 2 and 7). Those animals which received larger doses of replacement rations in the form of 1 per cent alfalfa in their basal diet (Table II) showed no radiographic changes.

Group III. A radiographic study was not made.

HISTOLOGIC FINDINGS

Our findings confirm in the main those of Wolbach and Howe.[2] The emphasis in this report will therefore be placed on those findings which supplement theirs.

Group I. Vitamin A Deficiency Without Replacement Therapy

The alterations seen in the roentgenograms were readily confirmed in the microscopic sections.

Subgroup A. Rats that Were Placed on the Experimental Diet for 24 or 26 Days Subsequent to Weaning. The newly formed lingual dentin was narrower than normal and irregular in its pulpal border. The adjacent odontoblasts were distorted. They were not columnar and had not completed their differentiation from the peripheral pulpal mesenchymal cells. The enamel epithelium was normal except for some minor hypoplastic changes in the proximal portion. The labial odontoblasts and the labial dentin were still normal. The odontogenic epithelium showed no morphologic alterations.

Subgroups B, C and D. Rats that Were Placed on the Experimental Diet for 34 to 52 Days After Weaning. The typical changes described below progressed with the increase in the survival period and varied only in degree. The disturbances were more severe in the proximal than in the distal portions (Fig. 10).

The characteristic alterations in these groups follow. Since there was a sharp difference between the changes in the enamel-covered and the cementum-covered portions of the incisor, the corresponding changes will be described separately.

Enamel-Covered Portions of Incisor

Disturbances in Formation

Enamel Epithelium. The epithelial papillae of the enamel organ showed occasional distortions but the changes were not prominent except in the hypoplastic areas. At the level of the alveolar crest, the papillary arrangement was, as a rule, still present. Sometimes the papillae proliferated and gathered in masses which gave the appearance of peninsulae of stratified squamous epithelium. In such cases the ameloblasts had become low or flattened or had lost their identity. Enamel hypoplasia was quite severe and common. Degenerating epithelial cells which in some instances had undergone calcification were often found in the hypoplastic crypts (Fig. 20). The ameloblasts showed premature atrophy only in the cases of longest experimental survival.

Enamel. The organic enamel matrix, which normally terminates at the distal end of the proximal third of the tooth (about 7.5 mm. from the odontogenic base), was found to terminate between 50 and 200 μ from the odontogenic base (Figs. 9 and 10). In the longer survivals the organic enamel matrix and the corresponding dentin were often buckled and wavy in the extreme proximal portion (Fig. 10).

This picture reminds one of that seen with long survival after hypophysectomy.[17] In hypophysectomy, however, the foldings were deeper and more numerous. Often, concurrently with these violent disturbances, a large vesicular area was observed near the proximal end of the organic enamel matrix (Figs. 10 and 17). This was the vesicle described in the X-ray findings. It communicated with the pulp and was lined peripherally with normal odontoblasts. The lumen was filled with pulpal cells and an occasional island of epithelium and osteodentin.

Labial Dentin. This dentin was wider than normal (Table V), but for the most part normal in structure and in staining reaction. In the proximal region the odontoblasts as a rule showed no morphological disturbances. In the distal region they attained a cuboidal and finally a spheroidal form and resembled osteoblasts. They appeared to have lost their attachment to the dentin and to have migrated toward the center of the pulp. Here, islands of osteodentin with cellular and vascular inclusions were abundant. In some of the animals of longest survival the odontoblasts showed alterations even in the proximal region.

Disturbances in Calcification

In some animals the dentin showed to a marked degree interglobular dentin which was accompanied by an abnormal width of predentin (35 to 50 μ) at the midthird level. It was found that these animals had received a basal diet which was less fortified with vitamin D than usual. These animals showed in addition fibrotic changes in the pulp that resembled scar tissue and that were more prominent than those found in the other experimental animals (Fig. 26).

Cementum-Covered Portion of the Incisor

Disturbances in Formation

Dentin. The normal histologic characteristics of the lingual dentin were lost. In animals of shorter survival the changes were

confined chiefly to the proximal third of the tooth. After longer survival the disturbances extended through the entire length of the lingual dentin (Fig. 10). The dentin was much narrower than normal at any particular level. Thus, at the midlingual level the width was one half the normal width or even less (Figs. 2 and 14, Table V). Its pulpal surface was irregular. The matrix frequently lacked dentinal tubules and resembled osteodentin. It contained scattered cellular inclusions and occasional vascular inclusions. The latter, however, occurred consistently near the cemento-enamel junction (Fig. 12). The odontoblasts were disorganized and showed the most severe disturbances near the proximal end. They often assumed a spheroidal outline. Osteodentin was deposited either along the lingual pulpal wall or in the pulp (Fig. 13). In the more advanced cases the dentinocemental junction was disturbed (Fig. 12). The proximal end of the lingual dentin was situated more distally than normal (Fig. 10).

Disturbances in Calcification

The normal incremental calcification rhythm was absent (Figs. 11 and 12). The predentin was lacking and the dentin when stained with hematoxylin and eosin often took only the eosin color (Fig. 16).

Pulp. The pulp was displaced lingually, confirming the roentgenographic findings (Figs. 10 to 12). The most striking change was the invasion of epithelium which arose from the lingual odontogenic epithelium. Long cords of epithelium resembling in structure the lingual odontogenic epithelium of Hertwig's sheath extended into the pulp and continued to proliferate distally (Fig. 11). When these cords were cut transversely they gave the appearance of epithelial clusters or glandular acini. The cells were usually low cuboidal but sometimes assumed a columnar shape and arranged themselves radially (Fig. 16). The distal extent of these epithelial proliferations varied with the length of the survival. In some instances definite degenerative changes were observed which simulated thymic corpuscles (Fig. 19) and approached calcification. Osteodentin at times formed about the epithelial islands. The osteodentin was bordered by mesenchymal cells which were cuboidal in structure (Fig. 18). In longer survivals cauliflower-like islands of poorly calcified tissue projected

consistently from the lingual dentin into the pulp. They reminded one of the osteoid proliferations seen in rachitic compensatory hyperplasia. Encircling these islands and often caught in the meshwork of the matrix were found hematoxylin-staining cells which were spheroidal in shape and which differed markedly from the normal columnar-shaped odontoblasts (Figs. 11 and 13). The pulpal wall was very irregular and bayed as a result of the incomplete fusion of these islands. From these bays and irregularities many vascular inclusions dipped inward and penetrated the dentin for quite a distance. These inclusions often branched within the dentin matrix (Fig. 12).

The blood supply of the pulp was prominently reduced on the lingual surface. The connective tissue of the pulp tended to lose its normal embryonic-like character and became more fibrous, especially near the epithelial cords (Fig. 26). Calcospherites were not common.

Cementum. The cementum, which normally assumes a maximum width of 3 to 4 μ, often approached a width of 10 to 15 μ. The staining reaction with hematoxylin was pale. In occasional areas of communication between the pulp and the periodontal membrane the adjacent cementum was excessively wide, as if to compensate for the lack of attachment and cementum at the points of communication. Here, in place of the cementum, fibrous condensations of connective tissue tended to bridge the gap (Fig. 12).

Periodontal Membrane. In the normal rat the outline of the periodontal membrane, both on the cementum and the alveolar side, is fairly regular. Its width varies between 240 and 275 μ in the proximal half and between 100 and 125 μ in the distal half. In vitamin A deficiency the outline of the periodontal membrane was very irregular. The width varied considerably (40 to 200 μ) and in general was narrower than normal (Figs. 9 and 10).

Labial Alveolar Periosteum. The labial alveolar periosteum was considerably widened. It contained subacute inflammatory cells, crystals and fibrinous strands similar to those seen in clotting blood (Fig. 13). With late survival the blood supply of the periosteum seemed to be reduced.

Alveolar Bone. The socket bone (surrounding proximal base) appeared to be thicker than normal. The spicules in the bone

were prominent and very long, with the cementing lines staining readily with hematoxylin. The spicules were arranged regularly and parallel with the long axis of the incisor.

Transverse Sections. In cross sections two prominent and characteristic vascular inclusions extended along the dentinal tubules and coursed from the mesial and distal cemento-enamel junctions to the pulpal wall. It is interesting to note that the greatest disturbances of the tooth were located lingual to these two outstanding vascular inclusions (Fig. 12).

Transverse sections also indicated clearly the extreme distortion of the growth pattern that is characteristic of vitamin A deficiency. The pulpal surface which normally parallels closely the outline of the dentino-enamel and dentino-cemental junctions showed no order or regularity (Fig. 12). The dentino-cemental surface was also distorted and occasionally in localized areas the pulp and periodontal membrane communicated. In these areas fibrous bands of connective tissue tended to bridge the perforation and wall off and separate the pulp from the periodontal membrane. The cementum adjacent to the perforated areas was thickened (Fig. 12).

Group II. Vitamin A Deficiency with Replacement Therapy

Subgroup A. These animals which were given total replacement with 1 per cent alfalfa concurrently with the basal diet showed no abnormal changes in the incisors (Table II).

Subgroup B. The animals in subgroup B which were given replacement with a suboptimal amount of alfalfa during the last 8 weeks, following a period of complete vitamin A deficiency, showed histopathological disturbances which were similar to those observed in subgroups B, C and D of group I. The premature atrophic changes in the enamel organ were more evident in this group than in group I, probably because of the longer survival period.

Subgroups C and D. The teeth of those animals which were given full replacement therapy for 10 to 20 days showed evidence of a resumption of normal histodifferentiation. The peripheral mesenchymal cells of the pulp, which are responsible for dentinogenesis, and the new matrix in the cementum-covered dentin, which appeared since the institution of replacement and

which is found first at the proximal base of the tooth, were normal. The changes which had occurred prior to replacement therapy were still present in the middle and distal thirds of the tooth. These areas, however, showed a greater tendency toward fibrosis of the injured or malformed tissues than in animals at deficiency levels.

In the 10-day-replacement animals a spearlike projection of dentin extended obliquely from the linguo-proximal base into the pulp. This projection of dentin was bordered on the pulpal surface by normal odontoblasts. The dentin and predentin were of normal texture and showed a normal staining reaction (Fig. 24). The width of the dentin was 110 μ. Assuming that it was apposed at the normal daily rate of 16 μ, it was estimated that the new dentin had been apposed for approximately 7 days before death. In other words, apposition of new dentin had begun within the third day of the replacement period.

The form of the ectopic dentin laid down during early replacement depends upon the arrangement of the epithelial islands that had invaded the pulp during the deficiency. In the case of a spearlike epithelial invagination (Fig. 25) the newly formed dentin assumed a spearlike pattern (Fig. 24). Transverse sections of the newly formed dentin showed isolated circular areas of dentin with epithelial cells in the center (Fig. 18). These cells evidently regained their capacity to cause adjacent cells to differentiate into odontoblasts but did not acquire any amelogenic capacity. In no case was enamel or enamel-like tissue found in the pulp.

The changes during the 15 to 20 day repair period were similar to those for the 10 day period but more advanced.

Group III. The Effects of Vitamin A Deficiency Studied by Means of Vital Staining with Alizarin Red S

The data for this group were obtained from ground sections which were necessary for the determination of the rate of apposition since the alizarin effects were lost during decalcification. The findings, other than those due to alizarin, were those characteristic for the corresponding degree and duration of vitamin A deficiency.

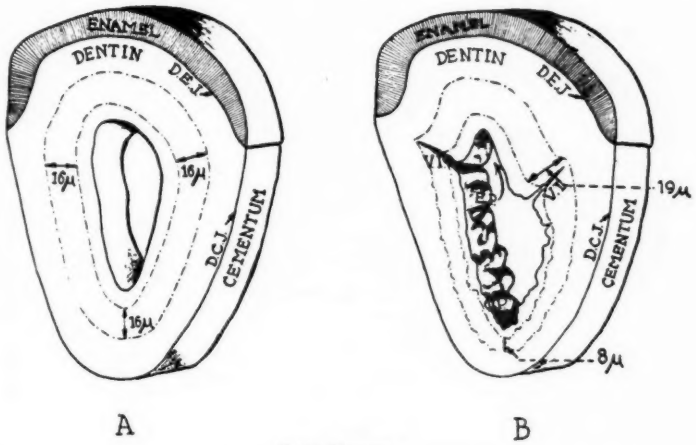
Injections of alizarin red S produced red lines which were superposed on the daily incremental rings which were being ap-

posed at the time of the injections. The rings were more intensely stained, sharp and distinct in the enamel-covered dentin, but wavy and relatively faint in the cementum-covered dentin. The course of the alizarin rings, in cross section, paralleled the outline of the pulpal wall and showed indentations with each vascular inclusion. The distance between the effects of any two injections on the labial aspect, from the cemento-enamel-junction level on the mesial to the corresponding level on the distal portion, was fairly parallel. The distance between the two lines was found to be much smaller immediately lingual to the cemento-enamel junctions, and sharply diminished toward the midlingual region of the tooth, where the distance between the two rings and the entire width of the dentin was narrowest. (Text-Fig. 2, Figs. 22 and 23).

The daily rate of apposition of both the enamel-covered and cementum-covered dentin was found, on the basis of more than 200 measurements on 12 control animals, to range between 15.25 ± 0.51 μ and 16.12 ± 0.73 μ. These figures were not significantly different from the normal average daily appositional rate of 16 μ found in the dentin of normal rats in previous reports.[13] The differences in the normal daily rates in the labial and lingual portions are very slight, so that the ratio of the thickness of the midlabial and midlingual dentin is 1:1.

The disturbances in the rate of appositional growth were manifest between 9 to 19 days following institution of vitamin A deficiency, long before the cytologic and histologic changes become apparent (Table III). The daily rate of apposition of enamel-covered dentin was found to be greater than normal and increased up to 19.63 μ, as the duration of vitamin A deficiency increased (Table III, Text-Fig. 2). The daily rate of apposition of cementum-covered dentin was less than normal and decreased as the survival period increased. In addition to this survival or age gradient, apposition also followed a locus gradient which decelerated uniformly from the cemento-enamel junction where it was relatively highest to the midlingual level where it was lowest (6.43 μ) (Table III).

Suboptimal replacement therapy of 4 days' duration did not produce measurable changes in appositional rates. However, full replacement therapy had an immediate effect, causing the normal



TEXT-FIGURE 2
(From Schour, Smith, and Hoffman: *Proc. Soc. Exper. Biol. & Med.*, 1938, 39, 448)

A. Microprojector tracing of section shown in Figure 22 from a normal control rat which was given intraperitoneal injections of 0.5 cc. of a 2 per cent solution of alizarin red S on the 50th and 60th days of life and sacrificed on the 65th day. Note that the alizarin red S effects (dotted lines) are parallel. The outline of the pulpal wall is regular, smooth and closely parallels the outline of the dentino-enamel (D.E.J.) and dentino-cemental (D.C.J.) junctions. The rate of apposition approximates 16 μ per day. × 71.

B. Microprojector tracing of section shown in Figure 23 of a vitamin A-deficient rat which was put on the deficiency diet on the 21st day of life; given intraperitoneal injections of 0.5 cc. of a 2 per cent solution of alizarin red S on the 50th and 60th days and sacrificed on the 65th day. Contrast with A and note (1) the greater thickness of the enamel-covered dentin; (2) the narrowness of the cementum-covered dentin; (3) the irregular and distorted pulpal outline with the deep vascular inclusions, V.I., at the cemento-enamel junctions, C.E.J. The pulpal space, E.P., which is next to the enamel-covered dentin, is considerably narrowed in contrast to the pulpal space, C.P., which is next to the cementum-covered dentin. The alizarin red S effects are indicative of a marked gradient resulting from the increased daily rate of apposition in the enamel-covered dentin (19 μ) and the decreased rate of apposition on the cementum-covered dentin (8 μ). × 71.

rate of dentin apposition (approximately 16 μ per 24 hours) to be resumed within a period of 1 to 5 days.

By measuring the total width of dentin at various anatomic locations (Table V) and dividing it by the estimated average daily rate of apposition it was found that while the rate of apposition in rats on vitamin A deficiency differed markedly from the normal, the life span of the formative cells showed little if any differences from the normal. The differences in the total width

of the dentin were thus due primarily to the differences in the rate of growth, and the ratio between the total thicknesses of the midlingual and midlabial dentin may be used as an index of the severity of vitamin A deficiency. The longer the duration of vitamin A deficiency, the greater the ratio between the daily rates or total dentin thickness of the midlingual and midlabial levels. Thus in the normal control, or in full replacement, the ratio is 1:1. During the second and third weeks following the institution of a vitamin A-deficient diet the ratio was 3:4; during the fifth and sixth weeks the ratio was 1:2, and during the seventh week, 1:3 (Table III).

Summary of the Effect of Vitamin A Deficiency on the Rate of Dentin Apposition. The rate of apposition of dentin was selectively altered in vitamin A deficiency, while the life span of the formative cells was not affected. The rate of apposition was accelerated in the enamel-covered portion and decelerated in the cementum-covered portion, with a uniform gradient effect. The extent of the deviation from the normal was in direct proportion to the duration of the vitamin A deficiency and may be expressed in the ratio between the total dentin width or daily rates at the midlingual and midlabial levels.

The Rate of Apposition of Dentin in Vitamin A Deficiency Followed by Graded Replacements. Table IV gives the experimental history and the quantitative findings in animals which were given graded daily replacement doses of cod liver oil following the depletion of the vitamin A reserves. One Sherman unit of vitamin A fed as cod liver oil did not measurably affect the rate of growth of dentin over that of the totally deficient animals (Table III). Two to four units, inclusive, had the effect of reëstablishing the labial rates to approximately normal. However, the lingual rates, though higher than in total deficiency, remained at a ratio of 3:4 to the labial rates up to the 23rd day following the institution of replacement. The five-unit replacement showed a resumption of normal rates of growth on both the labial and the lingual dentin by the tenth day following the beginning of replacement therapy.

FINDINGS IN MOLAR TEETH

Histologic Changes. The molar changes resulting from vitamin A deficiency were in direct proportion to the chronologic stage of

development of the tooth at the period when the vitamin A reserves were depleted in the animal. Since in the experimental animals the vitamin A body reserves were not exhausted until about the 45th day when the crowns and a considerable portion of the roots in the molar had completed their formation, the changes were slight in contrast to those observed by Mellanby,[12] in whose animals vitamin A deficiency was instituted in the maternal diet.

In our material the third molar, which is the last tooth to develop, showed a very irregular pulpal outline in the root portion. The odontoblasts were scattered or absent in localized areas. Projections of osteodentin were seen to grow out into pulp at various levels of the root. Epithelial islands were found near the pulp chamber and in the middle third of the root. Cysts were noted in several instances. In the first and second molars an abnormal number of epithelial pearls were present in the apical third of the roots (Fig. 27).

In animals of longer survival period (chronic deficiency) the changes were more severe. The dentin of the apical third as well as the last formed secondary dentin were irregular in their outline and amorphous in character.

Appositional Rates of Cementum and Alveolar Bone. Injections of alizarin red S produced in the first molar sharp, continuous red lines in the coronal and middle thirds, but distorted, irregular and discontinuous lines in the apical third. The irregularity of the alizarin lines in the secondary cementum similarly indicated an abnormal, amorphous formation. Table VI gives measurements of the daily rates of apposition of dentin, cementum and alveolar bone of the first molar. The rates were significantly lower than those in the molar of the normal rat.[18, 19] The width of the periodontal membrane was narrower than normal except for the fundic portion. The rate of eruption was retarded.

Discussion

Interrelationship Between the Odontogenic Epithelium and the Pulpal Mesenchyme. An analysis of the dental effects of vitamin A deficiency supported by recent studies in tissue culture [20] and transplant experiments [21] has thrown valuable light on the histophysiologic interrelationships and interdependencies of the various phases of tooth development. We may now reconstruct the

essential sequences of events and the interesting interplay between the remarkable organizing influence of the odontogenic epithelium and the responsive pulpal mesenchyme. This reconstruction is given in Text-Figure 1 and facilitates an understanding of the sequence of events in vitamin A deficiency.

THE EFFECTS OF VITAMIN A DEFICIENCY ON HISTODIFFERENTIATION

The characteristic dental changes point to the view that in vitamin A deficiency the primary and basic alteration lies in a disturbance of the odontogenic epithelium and specifically in the process of histodifferentiation of these cells. This interpretation is in accord with the view taken by Wolbach and Howe [2] and more recently by Wolbach.[3] Most of the other dental changes, such as the uninhibited proliferative growth of the odontogenic epithelium and the disturbances in appositional growth, may be regarded as secondary effects which are the resultants of a disturbance in histodifferentiation. In vitamin A deficiency the effect on histodifferentiation is evidenced by the following:

TABLE VI

*Measurements of Dentin, Cementum and Alveolar Bone Growth, and Rates of Eruption of First Molar for a Period of 50 to 65 Days in 10 White Rats Placed on Vitamin A Deficiency**

	Rates of apposition per 24 hours							Widths					Rates of eruption per 24 hours
	Dentin—tooth levels				Secondary cementum	Alveolar bone		Periodontal membrane				Secondary cementum	
								Root level in thirds			Fundus		
	Mid-crown	Cemento Enamel junction	Mid-root	Apical third		Fundus	Crest	Cervical	Middle	Apical			
	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)
Vitamin A deficient	4.2	3.6	1.7	1.3	9.8	4.9	7.2	52	58	104	125–130	52	14.8
Normal	5.2	4.5	2.8	2.4	12.4	7.0	11.4	102	118	131	122–129	174	19.7

* These animals were placed on vitamin A deficiency at 21 days of age, injected with alizarin red S on the 50th and 60th days and sacrificed on the 65th day of age.

1. Proliferative growth does not cease completely. Epithelial cells proliferate into the pulp. Wolbach and Howe [2] recognized the "acquisition of the neoplastic properties." The findings of Orten, Burn and Smith [10] of odontomas in prolonged chronic vitamin A deficiency may thus be explained.
2. The morphologic plan is disturbed. The outline of the dentino-enamel and dentino-cemental junction is distorted and often dysplastic.
3. The morphologic differentiation of the lingual odontogenic epithelium is incomplete.
4. The organizing influence of this epithelium upon the subjacent mesenchyme is thus incomplete.
5. The daily rate of dentin apposition is subsequently altered (increased rate on the labial and decreased rate on the lingual), although the life span of the cells appears to remain normal.

These changes represent experimentally induced accentuations or alterations of the following phases which normally occur in and closely follow histodifferentiation: [22]

1. Proliferation ceases. Histodifferentiation marks the end of the proliferative phase of cellular activity.
2. Establishment of the morphologic plan (the dentino-enamel and dentino-cemental junctions).
3. Morphologic differentiation of the cells (differentiation of cells of the inner enamel epithelium into ameloblasts).
4. Organization of adjacent mesenchymal cells by the epithelium.
5. Preparation for apposition which normally proceeds at a definite daily rate of activity during the life span of the cell.

The earliest specific effects upon the odontogenic epithelium are first recognized not in morphologic alterations but in functional behavior. The various functions of the odontogenic epithelium are not disturbed equally. The earliest effect is manifested in the linguo-lateral portion of the odontogenic epithelium which lacks the organizing principle that normally enables it to guide, stimulate and "organize" the mesenchymal cells of the pulp to differentiate into active dentin-forming cells. This capacity to organize mesenchyme is apparently much more severely disturbed in the lingual and latero-lingual portions than in the labial

portion of the odontogenic epithelium. In the latter, the capacity of the inner enamel epithelium to differentiate into ameloblasts is less severely disturbed.

Consideration of the Differences in the Reaction of the Labial and Lingual Dentin. The current knowledge of the normal embryologic and morphologic differences between the labial and lingual portions is not sufficient to explain the selective difference in the effects of vitamin A deficiency on the morphologic alterations and the rate of apposition. Wolbach and Howe [2] (1933) attributed to the enamel and the enamel organ a protective chemical function for the underlying labial dentin and its odontoblasts. While the decreased rate of apposition of the cementum-covered dentin is in harmony with the decreased body weight in vitamin A deficiency, the accelerated appositional rate of the enamel-covered dentin cannot be readily explained. A teleological consideration suggests that in the presence of a severe disturbance there is a compensatory thickening of the labial dentin which has to carry most of the functional stress in mastication.

Disturbances in Odontoblasts not Merely an Atrophy but a Lack of Normal Differentiation. The fact that in a given case the disturbances in the odontoblasts were more severe in the proximal than in the distal portions may be explained on the basis that the proximal tissue having been formed more recently was subject to the state of more advanced deficiency, associated with the longer survival period. It gives support to the interpretation that the odontoblasts are disturbed not because they have a lack of proper nutrition and thus atrophy, but rather because initially they are not given the opportunity to differentiate properly. The presence of normal odontoblasts in replacement therapy does not necessarily represent a recovery of atrophied odontoblasts. They rather appear to be new and young odontoblasts that have become differentiated from the mesenchymal cells under the influence of the now normally functioning odontogenic epithelium.

Disturbances in the Proliferative Growth of the Odontogenic Epithelium. Normally the proliferative phase of cellular growth becomes limited or ceases upon the assumption of histodifferentiation. It appears that in vitamin A deficiency the proliferative phase of growth of the cells of the odontogenic epithelium

is unchecked in proportion to their inability to reach the subsequent differentiative phase of growth. The lingual odontogenic epithelium shows prominent proliferation and invasion into the pulp. On the other hand, the labial odontogenic epithelium is much less disturbed in its histodifferentiation than is the lingual portion, and also shows relatively less proliferation into the pulp.

The Behavior and Fate of the Epithelial Cells in the Pulp. The continually proliferating epithelium appears to survive readily in the nutritive connective tissue of the pulp. Here it does not become a stratified squamous type of epithelium and shows no tendency to keratinization or cyst formation. On the other hand, there is a characteristic tubular arrangement which is suggestive of a glandular organ and which reminds one of the epithelial cords and rests observed normally in the periodontal membrane. Some of the proliferating epithelium, however, still possesses the chemotactic property to stimulate dentin formation. But this capacity is weak and defective so that the result is not a true dentin product but an irregular, amorphous and unorganized matrix.

In replacement therapy the organizing influence of the epithelium is regained. The adjacent mesenchymal cells become differentiated into odontoblasts and normal dentin is apposed within the pulp. The amelogenic capacity, in contrast, is not manifested even after replacement. The reason for this is probably the fact that the invading epithelium is derived chiefly if not entirely from the lingual odontogenic epithelium which even normally does not possess amelogenic capacity.

The amount of pulpal epithelium increases with the duration of the survival period. In chronic vitamin A deficiency extending over very long periods [10] the epithelium is found in the adjacent tissues. Signs of degeneration of the pulpal epithelium are not frequent although sometimes areas of hyalinization or nests that resemble Hassall's corpuscles (Fig. 19) are seen. The absence of epithelial cyst formation in the pulp and its presence in the periodontal tissue is also interesting. Huggins, McCarroll and Dahlberg [21] found that when isolated enamel epithelium was transplanted the epithelial cells did not become cystic but formed islands and cords of cells (with epithelial pearl formation).

Atrophy of Ameloblasts. The ameloblasts show less disturb-

ance than the odontoblasts. Premature atrophy of ameloblasts which, according to Wolbach and Howe,[2] represents the earliest response, was not observed in animals that were placed on vitamin deficiency for less than 60 days. Within this period we were unable to observe any deviation from the normal process. The rate of progress of cytomorphosis in the incisor of the adult rat is such that during normal eruption the normal atrophy and retrogression begin when the ameloblast has reached the incisal third of the tooth.[23] Premature retrogression of the ameloblasts was observed in animals that were on vitamin A deficiency for 82 days. This change, however, is not specific for vitamin A deficiency. It is readily found in vitamin B deficiency,[23] hypophysectomy,[17] parathyroidectomy [24] and magnesium deficiency.[25] Atrophy may also be found in a relatively more proximal position than normal in cases of retarded eruption. Here the ameloblasts will reach their stage of atrophy within the same approximate 50-day period, but their position in the anteroposterior direction, which is determined by the eruption rate, will be relatively more proximal than normal. In accelerated eruption, such as can be produced by cutting off the exposed portion of the incisors, the atrophy of the ameloblasts is not reached and their tall columnar appearance is retained even at the gingival crest.[26]

Vascular Inclusions. The vascular inclusions in vitamin A deficiency are characteristic and differ from those seen in parathyroidectomy.[24] In the latter condition the vascular inclusions recur at more or less regular intervals and penetrate the dentin in an almost straight line. The vascular inclusions in vitamin A deficiency are more numerous and branch within the matrix of the dentin.

In addition, especially deep vascular inclusions occur consistently at the cemento-enamel junction and represent a most severe alteration in dentin formation. In vitamin A deficiency the cemento-enamel junctions are critical sites where the growth gradients change suddenly from an increased daily rate above 16 μ to a decreased daily rate below 16 μ. The vascular inclusion appears to be a secondary effect following the premature cessation of odontoblastic growth.

To our knowledge, vascular inclusions in the human teeth at the cemento-enamel junction have not been reported. However,

it is not rare in human teeth to observe a vascular inclusion extending from the growth center to the pulpal horn. The incidence in human teeth and in the molars of the rat of pulpal inclusions below the growth centers may be explained on the basis of greater mechanical crowding of the formative cells with the result that some of them undergo atrophy and become embedded. It is possible that in the rat incisor the characteristic vascular inclusions at the cemento-enamel junction may also be due in part to a greater crowding of the odontoblasts. The cemento-enamel junction as seen in cross sections takes the form of an indentation. The change in the curvature of the dentinal tubules in this area gives further indication of the crowding of the odontoblasts.

The Effects of Replacement Therapy on the Rate of Apposition of Dentin. The findings demonstrate that dentin apposition, which was among the first processes to show manifestation of vitamin A deficiency, was also among the first to respond to replacement therapy. Thus dentin is not only a delicate recorder of alterations in calcium metabolism [27, 28] but also acts as a growth kymograph.

The findings in Table III show a selective response of the rate of dentin apposition to different degrees of deficiency (on the basis of the survival periods). These findings thus first suggested the possible use of this reaction as a biological method of measuring vitamin A content in foods. To test this possibility, a group of 35 rats was placed on total vitamin A deficiency and then given graded doses of replacement therapy (Table IV). The results showed significant differences in response to the daily administrations of 1 and 5 units of cod liver oil but showed no significant selective response to doses of 2 to 4 units.

It is evident that any attempt to utilize total vitamin A-deficient animals for a quantitative assay method for vitamin A content in foods would not be satisfactory. However, it is possible that a careful quantitative biologic assay for vitamin A content in foods may prove successful if normal animals, which would be put on a vitamin A-deficient basal diet plus graded doses of replacement, were used. It would then be of interest to see whether graded doses produce correlative gradients in appositional growth of dentin.

Summary and Conclusions

The effect of vitamin A deficiency upon the development of the incisor and molar teeth of the white rat was studied in 199 animals, in respect to alterations seen in roentgenograms and in histologic sections. Eighty-four of these animals were given various types of replacement therapy; 95 animals were subjected to vital staining with alizarin red S in order to study the rates of apposition of dentin.

The characteristic roentgenologic changes are described.

The histophysiologic findings were:

1. The primary effect of vitamin A deficiency is on the histodifferentiation of the odontogenic epithelium.
2. Histodifferentiation, particularly of the lingual odontogenic epithelium, is disturbed and incomplete, with the result that its normal *organizing influence* causing the pulpal cells to differentiate into odontoblasts is ineffective. The earliest response can be recognized in a morphologic and functional alteration of the lingual odontoblasts rather than in any morphologic change of the epithelium itself. The lingual dentin is abnormally thin.
3. Concomitant with the lack of histodifferentiation there is a continuation of the proliferative activity of the odontogenic epithelium. The result is an invasion of the pulp by epithelial cords which arise for the most part from the lingual odontogenic epithelium.
4. The morphologic outline of the tooth is distorted.
5. The rate of dentin apposition is selectively altered. The enamel-covered dentin shows an accelerated and the cementum-covered dentin a decelerated rate of apposition.
6. The pulpal epithelium has an aberrant organizing influence upon the adjacent mesenchyme which forms amorphous dentin.
7. Replacement therapy results in the resumption of the normal rate of dentin apposition and the prompt differentiation of the peripheral pulpal cells into odontoblasts.

Our findings confirm on the whole those of Wolbach and Howe,[2] although the duration and composition of our experimental diet was different.

The reaction in vitamin A deficiency offers ideal material for the analysis of a number of physiologic processes in tooth development.

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DESCRIPTION OF PLATES

PLATE 100

FIG. 1. Roentgenograms of right half of head of a normal control rat 43 days old. The calcified portions are traced in black. Compare with Figures 4 and 8. × 2.75.

FIG. 2. Roentgenogram of right half of head of an albino rat which was on a vitamin A-deficient diet for 81 days following weaning and was given 0.0075 gm. of alfalfa daily for the last 56 days. Note extreme thinning of lingual dentin; increased thickness of labial dentin; increased extra-alveolar length of the incisors; wide labial alveolar periosteum especially at basal zone; the sharp bend, pulpally, of the proximo-labial base; bleb on the labial surface of the upper incisor; dulled incisal bevels. Compare with Figures 4 and 8. Natural size.

FIG. 3. Roentgenogram of right half of head of a rat which was on a vitamin A-deficient ration for 49 days after weaning. Note position of the bleb in proximal zone and compare with Figures 2, 7 and 8 in which the survival was 81 days. The blebs in the longer survivals are located further distally. Natural size. (See Fig. 10.)

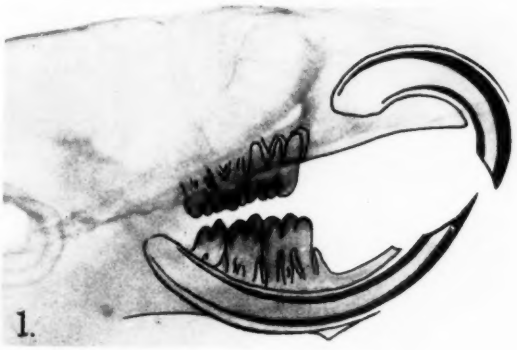
FIG. 4. Enlargement of roentgenogram of Figure 3 in which the calcified dental structures are traced in black. × 2.75.

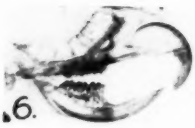
FIG. 5. Roentgenogram of right half of head of a rat which was on vitamin A-deficient ration for 56 days, but was given an optimum daily replacement in the form of alfalfa. Picture of roentgenogram is normal. Compare with Figure 1, and contrast with the other figures. Natural size.

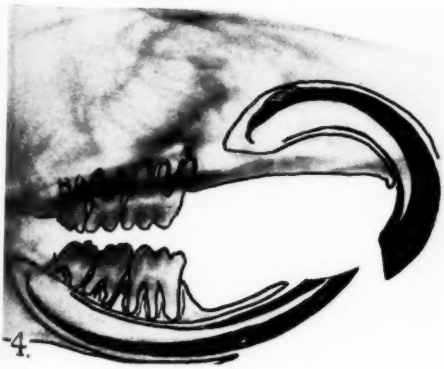
FIG. 6. Roentgenogram of right half of head of a rat which was on a vitamin A-deficient ration for 52 days following weaning. Note characteristic changes referred to in Figure 2. Natural size.

FIG. 7. Roentgenogram of right half of head of a rat which had a similar history as the animal in Figure 2. Compare with Figure 8. Natural size.

FIG. 8. Enlargement of roentgenogram of Figure 7, showing semidiagrammatic sketch of the incisor teeth. Note abnormal curvature and characteristic changes referred to in Figure 2. Compare with Figure 1. × 2.75.







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Incisor Teeth in Vitamin A Deficiency

PLATE 101

FIG. 9. A midsagittal section of an upper incisor of a normal rat 65 days old. Note the extension of the pulp to the distal edge and the relative thickness of the labial, D, to the lingual, d, dentin. Al.b. = alveolar bone; D = dentin; En.ep. = enamel epithelium; En.m. = organic enamel matrix; En.sp. = enamel space formerly occupied by enamel lost in decalcification; P.b. = proximal base of alveolar bone; P.d.m. = periodontal membrane. × 11.

FIG. 10. A midsagittal section of an upper incisor of an albino rat, 70 days old, which was on a vitamin A-deficient ration for 49 days after weaning. Note the relative thickness of the labial, D, to the lingual, d, dentin. En.hy. = enamel hypoplasia; En.m. = shortened organic enamel matrix; P.b. = thickened proximal bone; P.d.m. = narrowed periodontal membrane; V = vesicle on labial surface, characteristic for vitamin A deficiency. See Figures 3 and 4. × 11.

9.

10.

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FIG. 11. A midsagittal section of the upper incisor of an albino rat which was on the vitamin A-deficient diet for 81 days after weaning and was given a suboptimum dosage of alfalfa (0.0075 gm. daily) after the vitamin A reserve was depleted (25 days following the beginning of the deficiency diet). The animal was sacrificed at the age of 102 days. En.m.= abnormally shortened organic enamel matrix; En.ep. = enamel epithelium; En.sp. = enamel space; En.hy. = enamel hypoplasia; Ep.i. = epithelial islands which arose from the odontogenic epithelium, od.ep., and proliferated in pulp; L.a.b. = labial alveolar bone; D = interglobular and thickened enamel-covered dentin; L.a.p. = abnormally widened space of proximal labial alveolar periosteum; d = narrowing and abnormal morphology in the cementum-covered dentin; Od. = odontoblasts (lost on lingual); Osd. = osteodentin on lingual wall; P.osd. = peninsula of osteodentin extending into pulp. × 20.

FIG. 12. A cross section from the proximal third of the lower incisor of a rat which had the same experimental history as the animal in Figure 11. Note the prominent distortion of the tooth and the marked thickening and interglobular nature of the enamel-covered dentin, D. O and O′ indicate absence of cementum-covered dentin. Here the pulp communicates with the periodontal membrane, P.d.m., which is abnormally wide at the lingual aspect; v.i. = deep vascular inclusion which runs to the cemento-enamel junction, C.e.j.; Al.b. = alveolar bone; En.sp. = enamel space; d = atypical cementum-covered dentin; y = hypercementosis. × 45.

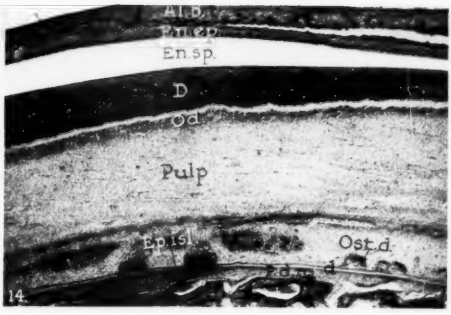
FIG. 13. A longitudinal section of the upper incisor of a rat which was on the vitamin A-deficient diet for 52 days after weaning. The animal was sacrificed at the age of 73 days. Note the thickened enamel-covered dentin, D. The organic enamel matrix, En.m., is shorter than normal and shows a definite area of hypoplasia, En.hy. At this level there is no dentin on the lingual to correspond with the formed labial dentin. Instead, a large accumulation of osteodentin, Ost.d., can be seen. (En.ep. = enamel epithelium which is still active in middle third of tooth; En.m. = abnormally shortened enamel matrix; L.a.p. = widened proximal area of labial alveolar periosteum containing large tissue spaces; Od. = odontoblasts of the labial pulpal wall.) No odontoblasts are seen on the lingual wall. × 30.

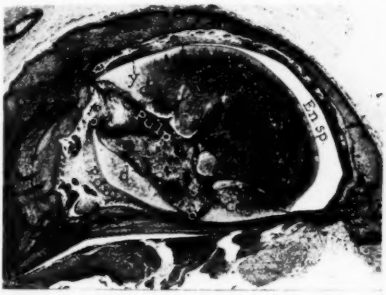
FIG. 14. A midsagittal section of the middle third of the upper incisor of a rat which was on a vitamin A-deficient diet for 50 days after weaning. Note interglobular and widened enamel-covered dentin, D; narrowed lingual dentin, d. (contrast with FIG. 15); islands of osteodentin, Ost.d., near lingual wall; epithelial islands, Ep.isl., in pulp near lingual wall; Od., labial odontoblasts. There is no evidence of lingual odontoblasts. Al.b. = alveolar bone; En.ep. = enamel epithelium; En.sp. = enamel space; P.d.m. = abnormally narrowed periodontal membrane for this level. × 45. Contrast with Figure 15.

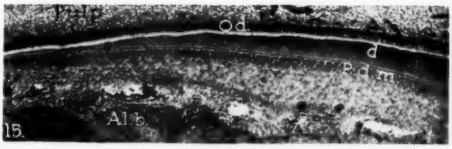
FIG. 15. A midsagittal section of the upper incisor of a normal rat illustrating normal cementum-covered dentin, d., and normal periodontal width, P.d.m. Note presence of odontoblasts which are lacking or deficient on the lingual wall in vitamin A deficiency. Contrast these conditions with Figure 14. × 90.

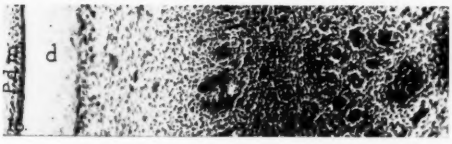
FIG. 16. A midsagittal section of the upper incisor of a rat with similar history as that used for Figure 12, showing high power field of the proliferating epithelial islands, Ep.i., in the pulp similar to those seen in Figure 11. Abnormal lingual dentin, d. P.d.m. = periodontal membrane. × 170.

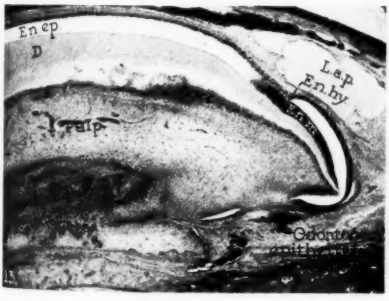
FIG. 17. A midsagittal section of the upper incisor of a rat which was on the vitamin A-deficient diet for 49 days after weaning, illustrating the histology of the vesicle seen in the roentgenograms (Figs. 3 and 4). En.ep. = enamel epithelium; En.sp. = enamel space; D. = interglobular enamel-covered dentin; Od. = odontoblasts; Ost. = osteodentin within vesicle and adjacent to enamel space. × 85.











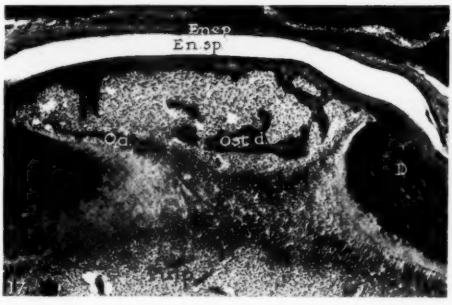


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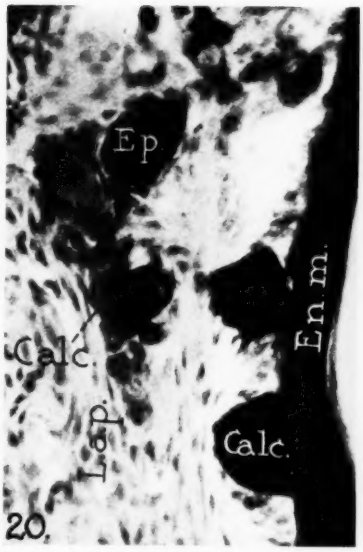
FIG. 18. A high power field of a section of the distal portion of the pulp of the upper incisor of the same albino rat as seen in Figure 13. A layer of atypical dentin, d, has been deposited by the pulpal cells, under stimulation from the epithelial cells, Ep., which arose from the odontogenic epithelium and proliferated in the pulp. M. = mesenchymal cells which have become only partially differentiated into odontoblasts. × 420.

FIG. 19. A high power field of the distal portion of the pulp of the upper incisor of a rat which had a similar history as that of Figure 18, showing the dense pulpal tissue, P.t. Some of the epithelial cells, Ep., have degenerated and resemble a Hassall's corpuscle. × 420.

FIG. 20. Enamel hypoplasia in a field from the proximal area of the upper incisor of a rat which was on vitamin A-deficient ration for 38 days after weaning. Note degenerating epithelium, Ep., which is giving rise to calcified masses or calcospherites, Calc. En.m. = organic enamel matrix; L.a.p. = connective tissue in labial alveolar periosteum. × 420.

FIG. 21. The distal third of the enamel organ of the upper incisor of a rat which was on vitamin A-deficient ration for 81 days after weaning, illustrating severe atrophic changes in the enamel epithelium, En.ep. The enamel papillae, P., show at this level greater atrophy than is normally present. En.sp. = space formerly occupied by enamel which was lost in the decalcification process; L.a.p. = connective tissue of the labial alveolar periosteum. × 700.



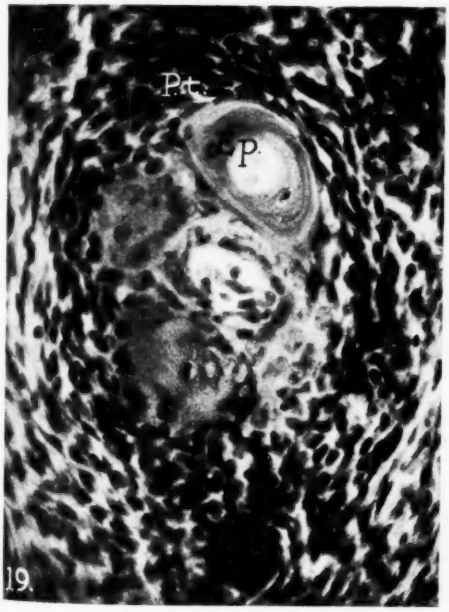


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Plate 104

Fig. 22. A transverse ground section of the lower incisor taken at the level of the mental foramen in a normal albino rat, 65 days old, which was given injections of alizarin red S on the 50th (I) and 60th (II) days. Note injection effects (I and II) and the uniform distance between them. Al.b. = alveolar bone; P.D.M. = periodontal membrane. See Text-Figure 2, A. × 90.

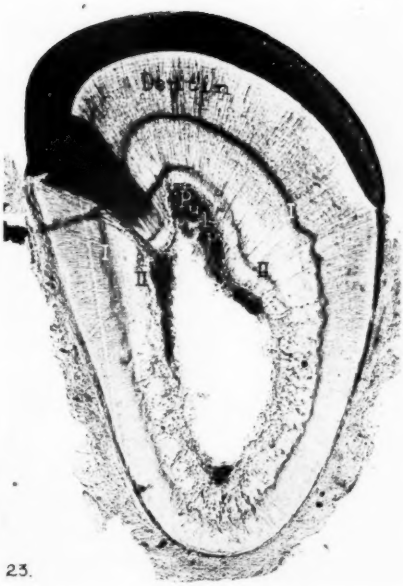
Fig. 23. A transverse ground section of the lower incisor taken at the level of the mental foramen in an albino rat, 65 days old, which was on a vitamin A-deficient diet since the 21st day of age. Injections of alizarin red S were given on the 50th (I) and 60th (II) days. Note injection effects (I and II) and the unequal distance between them. See Text-Figure 2, B. × 90.

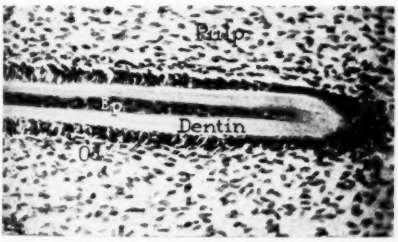
Fig. 24. A midsagittal decalcified section of the upper incisor of an albino rat, 60 days old, showing the linguo-pulpal area in the proximal region. This rat was on vitamin A deficiency from the 21st to the 50th day and placed on vitamin A therapy from the 50th to the 60th day of age. Note the center core of epithelium, Ep., which arose during deficiency as a pulpal invagination of the lingual odontogenic epithelium, and the dentin, which was deposited as a result of the differentiation of the approximating mesenchymal cells (odontoblasts), Od., following the institution of vitamin A therapy. Replacement effects were noted as early as the third day after the beginning of therapy. Contrast with Figure 25. × 280.

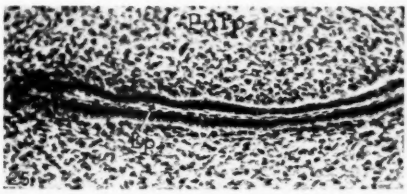
Fig. 25. A midsagittal decalcified section of the upper incisor of an albino rat, 60 days old, showing a field similar to that in Figure 24. This rat was on a vitamin A-deficient diet since its 21st day. Note the epithelial cord, Ep., which arose as a pulpal invagination of the lingual odontogenic epithelium, and the absence of mesenchymal differentiation and dentin apposition. × 280.

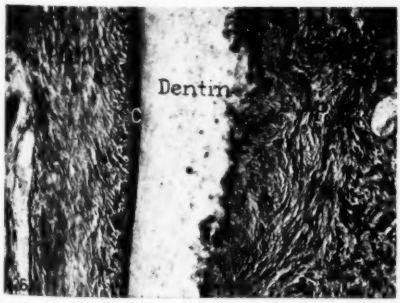
Fig. 26. The lingual middle third of a midsagittal section of the upper incisor of an albino rat, 65 days old, which was on a vitamin A deficient diet since its 21st day. Note the abnormal fibrotic texture of the pulp which adjoins the amorphous lingual dentin. C. = cementum; P.D.M. = periodontal membrane. × 178.

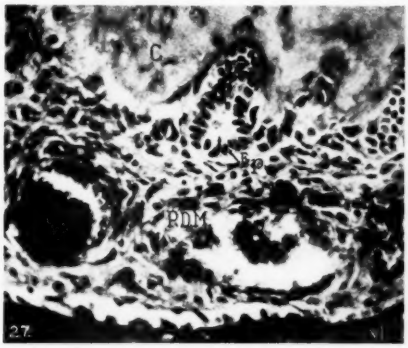
Fig. 27. The apex of the distal root of the lower first molar of an albino rat, 65 days old, which was on a vitamin A-deficient diet since its 21st day. Note the abnormally prominent epithelial pearl, Ep., which is being engulfed by the amorphous secondary cementum, C. P.D.M. = periodontal membrane; Al.b. = fundic alveolar bone. × 480.











Schour, Hoffman and Smith Incisor Teeth in Vitamin A Deficiency

THE OCCURRENCE OF MITOTIC DIVISIONS IN GLOMERULI IN GLOMERULONEPHRITIS AND MALIGNANT SCLEROSIS*

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The purpose of this publication is to prove that, contrary to current opinion, mitotic divisions in glomeruli in glomerulonephritis and malignant sclerosis are not exceptional. Though microscopic examination of the kidneys could be performed in but a small percentage of our material, yet in 3 of 30 cases thus examined from a series of 140 autopsies, mitotic divisions in glomeruli could be readily demonstrated in epithelial and endothelial cells.

MATERIAL AND METHODS

In cases Nos. 1 and 3, autopsy was performed 45 minutes after death, and in case No. 2, 30 minutes after death. Slices of renal tissue, not more than 3 mm. thick, were cut for fixation. In case No. 1, Stieve's solution [1] (saturated solution of corrosive sublimate, 76 cc.; undiluted commercial formaldehyde solution, 20 cc.; glacial acetic acid, 4 cc.) was used as fixative; in cases Nos. 2 and 3, a mixture of equal parts of a saturated solution of corrosive sublimate and of Bouin's solution. This mixture, recommended by Petersen [2] for embryological material, gives excellent results with surgical and autopsy material and is, in our opinion, much superior to Bouin's fluid alone. The material from cases Nos. 2 and 3 was embedded in paraffin after treatment with Peterfi's methylbenzoate-celloidin mixture.[3] In case No. 1 it was deemed necessary to avoid as much as possible the shrinkage often apparent in paraffin sections. Therefore, the tissues were dehydrated in glycerin,[4] soaked for 3 days in a mixture of five parts of a 6 per cent celloidin solution and one part of oil of cedarwood; hardened in anhydrous chloroform and thereafter embedded in paraffin after passing through benzene and benzene-paraffin. Sections were cut 5 and 6 μ thick and stained with azocarmine-aniline blue and hematoxylin-azophloxin.

* Received for publication December 21, 1940.

Reports of Cases

Case 1

A colored girl, 16 years old, entered the hospital 3 weeks after giving birth to a full-term infant. The only anamnestic data available were that she had had convulsions. The patient died a few minutes after admission, so that clinical data are not available.

Gross Notes. Autopsy was performed 45 minutes after death. The most important findings were edema of the lungs, possible slight enlargement of the heart (270 gm.) and lack of significant changes in the internal genitalia. The kidneys weighed 340 gm. The capsules stripped very easily and the surface of the kidneys was pale, smooth and showed very few small hemorrhages. On section the cortex and medulla were well delimited, the medulla being a little darker than the cortex, and the glomeruli were enlarged, pale and plainly visible. The renal pelves showed nothing abnormal.

Microscopical Examination. The glomeruli were enlarged and very cellular. This cellularity was partly due to the presence of polymorphonuclear leukocytes, amongst which several eosinophilic leukocytes were found. The epithelium of the visceral layer of the capsule of Bowman was visible only on the outer surface of the glomerular loops and showed only a slight swelling and sometimes a few hyaline droplets. The outer basal membranes were clearly visible. The inner basal membranes were fused in many places; in others they were lying in close contact but were still visible as two separate membranes. In some places individual capillaries, sometimes containing a few erythrocytes, could still be distinguished. Many capillaries were fused and between the endothelial cells a surprisingly great number of very delicate, often branching, fibers were found. The fibers were much more delicate than those depicted by Bell [5] and could not be called hyaline. Real hyalinization was absent. It was interesting to note that in ordinary paraffin sections of the same material, stained in the same way, the number of the individual fibers seemed much smaller than in the material first embedded in celloidin, whereas in the paraffin sections the closely adjacent basal membranes were distinguished with great difficulty. In the much reduced capsular spaces no red blood cells or fibrin were found.

In many glomeruli, mitotic divisions were found, sometimes three in one glomerulus. The distribution of the mitoses over the different glomeruli was unequal. In some sections many glomeruli had to be examined before a mitosis was found; in other sections they were detected quickly and easily. The mitotic divisions were found in all stages, ranging from the spirem to the diaster. With the azocarmine stain the centrioles and achromatic spindles were easily demonstrated, so that it was impossible to mistake dark-staining, degenerating nuclei of leukocytes for mitotic figures. In some cases, as illustrated in Figures 1 and 2, the dividing cell could, by its location, be recognized as an endothelial cell, as it was lying just inside the outer basement membrane. In the epithelial cells mitoses could not be found. The tubules contained little granular material and the epithelial cells were swollen and sometimes showed hyaline droplets. Only in very few tubules were red blood cells found. The interstitial tissue was unchanged.

Epicrisis. This is a typical example of acute glomerulonephritis showing intracapillary fibers, polymorphonuclear leukocytes and proliferation of the endothelial cells. In the glomeruli typical mitoses could be demonstrated and in several instances the dividing cell could be recognized as endothelial.

Case 2

A colored man, 40 years old, entered the hospital and died 1 hour after admission. Clinical and anamnestic data were not available.

Gross Notes. Autopsy was performed 30 minutes after death. The most important findings were as follows: The kidneys were enlarged, the left kidney weighing 365 gm. and the right 315 gm. Their consistence was diminished, the capsule stripped easily and the surface was smooth and yellowish gray. On section, the cortex was widened, welled up above the cut surface, and its color was gray-yellow, with deeper yellow streaks. The liver was enlarged, weighing 2300 gm., and was yellowish light brown in color, with small yellow spots. The spleen was enlarged, weighing 285 gm., and showed on section many yellowish white spots on a red background. The heart was not enlarged and weighed 245 gm. The heart muscle was brown-red. Retroperitoneal lymph nodes were swollen, moist, and whitish. There was only slight edema.

Microscopical Examination. The glomeruli were not enlarged. The basal membranes in nearly all glomeruli showed thickening, which was in general not very pronounced but was more strongly developed in individual glomeruli or in some loops of a glomerulus. Sometimes it appeared that the basement membrane, especially in the peripheral parts of the loops, was split longitudinally and surrounded the endothelial cells. Also, that small short fibers split off from the basement membrane. The endothelial nuclei were distinctly increased. The epithelial cells of the visceral layer of the capsule of Bowman were very conspicuous. Their protoplasm and nuclei were swollen and they often contained vacuoles and hyaline droplets. In several glomeruli, mitotic divisions were found and it could easily be ascertained that the dividing cells were lying outside of the basement membrane and belonged to the visceral layer of the capsule. The tubules were often widened and contained granular and hyaline casts. Many epithelial cells were swollen and showed hyaline droplet degeneration; in other tubules the epithelial cells were flattened. In the epithelium of the tubules many mitotic divisions were found. The interstitium was edematous and contained very few lymphocytes and some histiocytes.

Epicrisis. On purely morphological grounds we believe this case to be an instance of glomerulonephritis closely related to lipoid nephrosis (Bell [6]). Mitotic divisions were found in the epithelial cells of the glomeruli.

Case 3

A colored man, 37 years old, entered the hospital complaining of dullness, headache which had been increasing for several days, and vomiting of blood. On admission the heart was enlarged and the pulse rate was 125 per minute. Examination of the ocular fundi was impossible. The blood pressure was 170 systolic and 120 diastolic. Examination of the urine showed the specific gravity to be 1006; albumin and glucose, negative; urobilin, positive; acetone, negative; red blood cells, none; casts, none. The blood showed the hemoglobin to be 65 per cent; red blood cells, 3,900,000; sedimentation rate, 54 to 86 mm. (Westergren); Wassermann's and Kahn's tests, negative; nonprotein nitrogen, 300 mg. per cent, and blood urea, 280 mg. per cent. The electrocardiogram showed a serious myocardial lesion, probably a bundle-branch block. Tests of kidney function could not be performed.

On the day of admission the patient vomited a few black coagula and some fresh blood. Thereafter the vomiting of blood stopped, but the condition of the patient deteriorated rapidly. On the fourth day Cheyne-Stokes' breathing appeared and the patient died 7 days after admission.

Gross Notes. Autopsy was performed 45 minutes after death. The principal findings were as follows: The heart was enlarged, weighing 545 gm. There was extensive necrosis in the wall of the left ventricle and in the papillary muscles, less extensive in the wall of the right ventricle, and coronary sclerosis, especially of the ramus descendens anterior sinister, of which the lumen was very much reduced. Typical syphilitic lesions were present in the aorta and in the innominate and subclavian arteries. The kidneys were enlarged, the right and left kidneys weighing respectively, 255 and 215 gm.; their consistence was diminished, the capsules stripped easily, the surface was nearly smooth and the color was grayish brown with the admixture of some yellow, showing, in addition, very numerous irregular red spots of the size of a pinhead, sometimes even a little larger. On section the cortex welled up, the demarcation between cortex and medulla was not distinct and many small irregular red spots were visible. The pelvis showed nothing of significance. The blood vessels on section did not project and their lumina were open. No gross lesion, which could have caused the vomiting of blood, was found.

Microscopical Examination. Most arterioles showed an extensive hyalinization and fatty degeneration. In many places arteriolonecrosis or endarteritis was observed. The glomeruli showed a variety of lesions; glomerulonecrosis, hemorrhages, necrosis of some capillary loops and in many instances alterative or proliferative glomerulitis. Others showed a progressive hyalinization, although still others appeared quite normal. In the glomeruli showing alterative or proliferative glomerulitis, the epithelial cells were swollen and sometimes giant cells with three or more small nuclei were found. Hyaline droplet degeneration was quite common. In the glomeruli with proliferative glomerulitis the endothelial nuclei were increased. Mitotic divisions were found in epithelial and endothelial cells. Many tubules were filled with erythrocytes or with hyaline or granular casts. Hyaline droplet degeneration was quite frequent. Small areas with atrophic tubules and infiltrates of lymphocytes were found.

Epicrisis. We believe this to be a case of malignant sclerosis, complicated by luetic aortitis and infarction of the heart. That the blood pressure was not so high as usual was probably due to the cardiac failure. In the glomeruli, mitoses were found in both endothelial and epithelial cells.

Discussion

As Bell [5] stated, it is generally agreed that the essential lesion in glomerulitis is an increase in the number and size of the endothelial cells. On the other hand, most authors agree on the absence of mitotic divisions in the endothelial cells; neither are mitoses in the epithelial cells mentioned in recent publications. Bell therefore concluded that if cell division actually occurs, it is largely of the amitotic type. As experienced cytologists either consider amitosis very rare in mammals (Levi [7]) or doubt the existence of real amitosis (Maximow and Bloom [8]), this is not a satisfactory solution of the problem. Other authors state simply that there is an increase in the number of the endothelial cells (Kimmelstiel and Wilson [9]) without telling how this increase is brought about, and this is true also of many textbooks (Aschoff,[10] Hueck,[11] Fishberg,[12] Hadfield and Garrod [13]). Van Waveren [14] assumed that the endothelial nuclei always outnumber epithelial, though Bell [5] and especially von Möllendorff,[15] the latter using the most excellent histological technic, came to directly opposite conclusions. As one of us worked in the same laboratory as van Waveren and performed part of the autopsies from which he obtained his material, we are in a position to confirm Bell's [5] opinion that the material and methods of van Waveren were quite unsuited for these investigations. Only Kaufmann [16] records the finding of mitoses in "adventitial cells" of the capillaries of the glomeruli.

It is a well known fact that after death the number of mitoses found in a given tissue decreases with time (Schmorl,[17] Mallory[18]) and it is therefore not surprising that in tissues, fixed many hours after death, the number of mitoses found may be small, even in rapidly growing tissues. Furthermore many mitoses become indistinct (Casey [19]) and it is difficult to distinguish them from the nuclei of degenerating cells. In connection with this it is interesting to note a quotation from Karsner, Saphir and Todd,[20] by MacMahon,[21] who was the first to describe the regeneration of heart-muscle fibers in infants. Failing to find mitotic figures in hearts of adults these authors remarked that this was perhaps due to the fact that the hearts were obtained *post mortem*.

In our cases the autopsies were performed a very short time after death, thin slices of tissue were fixed and rapidly penetrat-

ing fixatives were used. We believe that these factors enabled us to find the mitotic divisions. When we compare sections of other organs from our autopsy material, 50 per cent of which is fixed less than 1 hour after death, with those from other laboratories in which we have worked and where the interval between death and autopsy averaged more than 24 hours, the difference in the number of mitotic divisions is striking. The same is true of surgical material instantly cut into thin slices and fixed after removal from the body as compared with large specimens, often whole organs or large tumors, when left untouched for some time or placed in their entirety into a fixative. Such material is, of course, quite sufficient for diagnostic purposes but is not suitable for delicate histological work, a fact often forgotten by pathologists.

Summary

A brief description of one case of acute glomerulonephritis, one case of subacute glomerulonephritis and one case of malignant sclerosis is given. In the first case mitotic divisions were found in the endothelial cells of the glomeruli, in the second case in the epithelial cells of the glomeruli, in the third case in both endothelial and epithelial cells.*

The importance of early and good fixation for the study of the glomerulus, and especially for the finding of mitotic divisions, is stressed.

* After submitting this paper we examined the kidneys of a man dying from septicemia with widespread metastatic abscesses. A few mitotic divisions were found in the endothelial cells of the glomeruli. The autopsy was performed 5 minutes after death.

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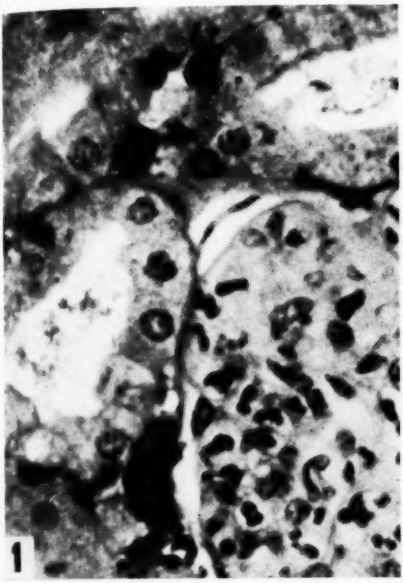
DESCRIPTION OF PLATES

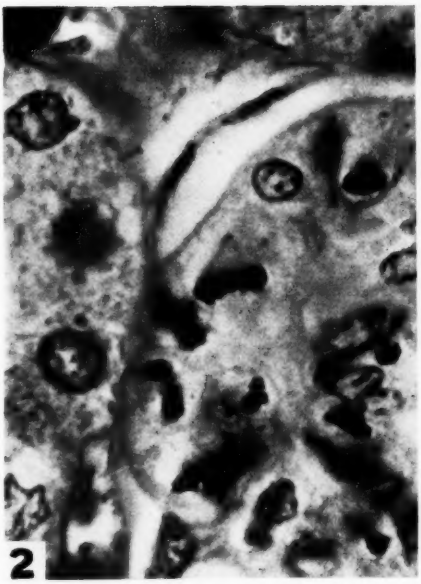
Plate 105

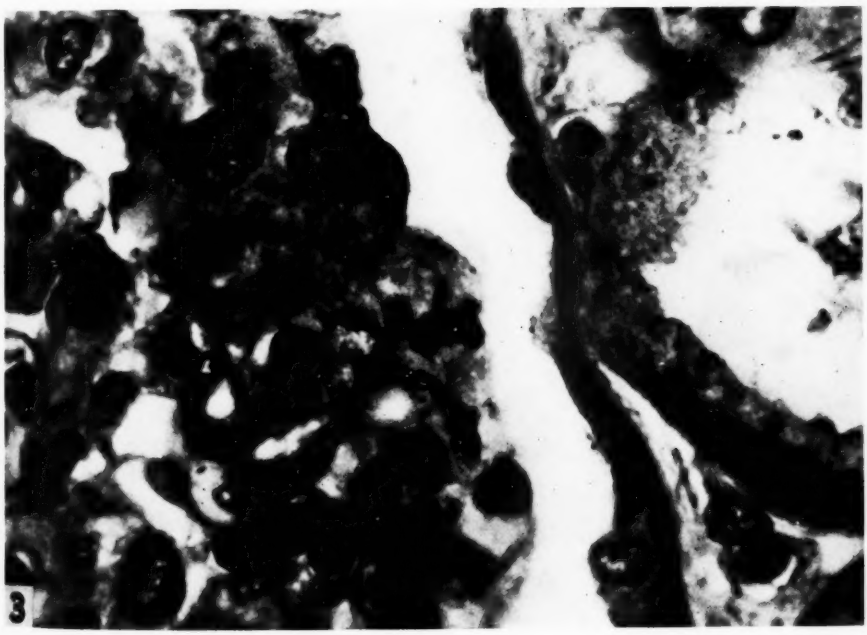
Fig. 1. Case 1. A glomerulus with a mitosis in its upper left corner and in the center of the field. × 716.

Fig. 2. Case 1. The same mitosis at a higher magnification. The dividing cell is lying inside the basement membrane and must be considered as endothelial. One centrosome is visible. × 1500.

Fig. 3. Case 2. A mitosis in an epithelial cell. Centrosomes and spindles are plainly visible. × 1500.







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Mitotic Divisions in Glomeruli

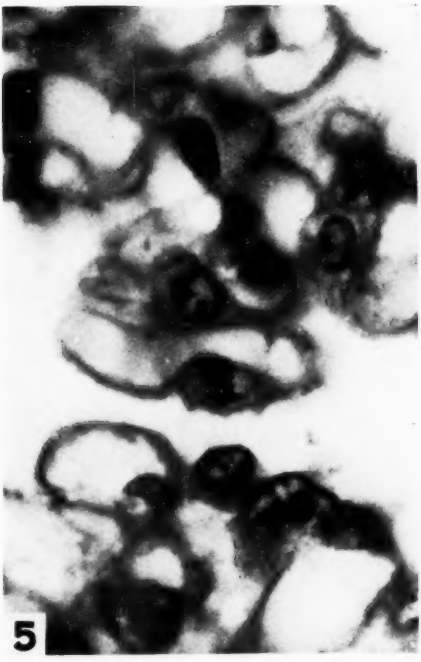
PLATE 106

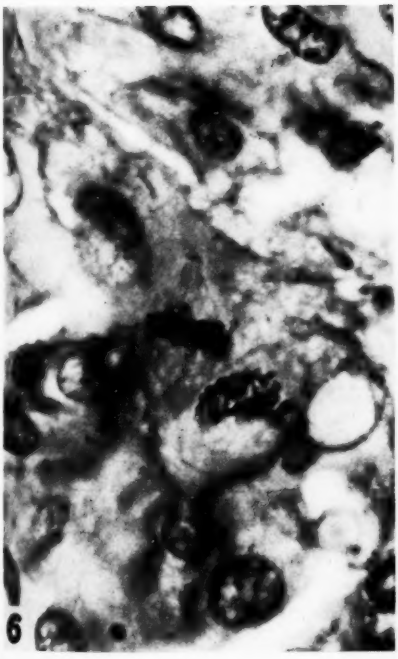
FIG. 4. Case 2. A mitosis in an epithelial cell. × 1500.

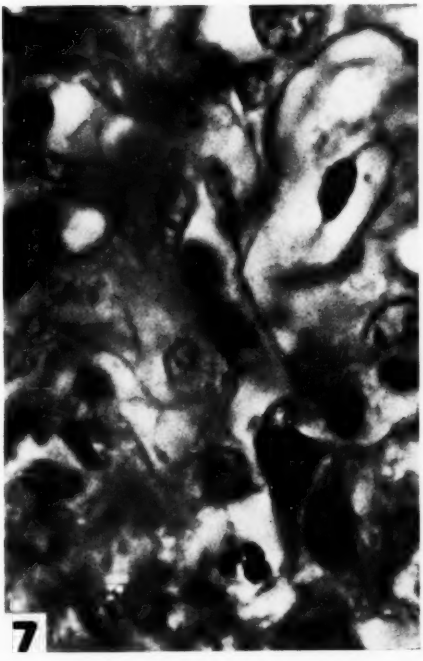
FIG. 5. Case 3. A mitosis in an endothelial cell. × 1500.

FIG. 6. Case 3. Two mitoses in epithelial cells at the vascular pole of a glomerulus. × 1500.

FIG. 7. Case 3. A diaster in a glomerulus. × 1500.







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Mitotic Divisions in Glomeruli

FORTY-FIRST ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

NEW YORK CITY

APRIL 10TH AND 11TH, 1941

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-First Annual Meeting, New York University Medical College, New York City

April 10th and 11th, 1941

PRESIDENT BAYNE-JONES IN THE CHAIR

BUSINESS MEETING

April 10th, 1941 .

On nomination of the Council the following officers were elected by the Association:

President	SAMUEL R. HAYTHORN
Vice-President	PAUL R. CANNON
Secretary	HOWARD T. KARSNER
Treasurer	ALAN R. MORITZ
Incoming Member of Council	ERNEST W. GOODPASTURE
Assistant Secretary	FRANCIS BAYLESS
Assistant Treasurer	GRANVILLE A. BENNETT

The Secretary announced the election of the following new members:

Lauren V. Ackerman
Joseph A. Beeman
Gerson R. Biskind
William C. Black
Mark M. Bracken
Lloyd Catron
A. Reynolds Crane
Malcolm B. Dockerty
Jesse E. Edwards
W. Norman Elton
Cyrus C. Erickson
J. Howard Ferguson
James S. Forrester, Jr.
Raymond Gettinger
Arthur M. Ginzler
Ralph M. Hartwell
Robert Hebbel
Edgar S. Ingraham
William Kaufmann
Donald H. Kaump
Simon Koletsky
Joseph R. Kriz
Herbert Lund
John C. McCarter
Anderson Nettleship
Lincoln Opper
Bjarne Pearson
Donald J. Rehbock
Seaton Sailer
Murray Sanders
George A. C. Snyder
Leon J. Tragerman
Jacob Werne

The Secretary announced the election of Tracy B. Mallory as Assistant Editor of the *American Journal of Pathology*, and J. Harold Austin to membership on the Editorial Board for a term of six years beginning January 1, 1942, to succeed H. Gideon Wells whose term expires December 31, 1941.

The Secretary announced that the Council had voted to issue a special number of the *American Journal of Pathology* in honor of H. Gideon Wells.

The Council recommends that in Article 2 of the By-Laws the sentence "The Council shall consist of seven Members elected by the Association, and the Secretary and Treasurer ex officio" be changed to read "The Council shall consist of seven Members elected by the Association, and the Secretary, Treasurer and Editor of the *American Journal of Pathology* ex officio."

Voted to accept with regret the resignations of R. L. Cecil, R. S. Cunningham, H. A. Kemp, H. McL. Kinghorn, F. A. McJunkin, Carl TenBroeck and R. L. Thompson.

Voted to record with deep regret the deaths of Maude E. Abbott, D. M. Cowie, O. I. Cutler, J. G. Fitzgerald, J. A. Lanford, David Perla, Ernest Pribram, D. G. Richey, Kurt Semsroth and Hans Zinsser.

The Secretary announced that the next meeting of the Association will be held as the guests of Washington University in St. Louis, Missouri, April 2 and 3, 1942.

The Secretary announced that it had been voted to have a symposium on "Neoplasms of Endocrine Glands" and to appoint Howard T. Karsner as referee.

The Secretary announced the election of N. Chandler Foot as delegate of the Association to the Executive Committee of the Congress of American Physicians and Surgeons and Irving Graef as alternate.

The Secretary announced the election of Samuel R. Haythorn as delegate of the Association to the Celebration of the Fiftieth Anniversary of the University of Chicago and of Louise Pearce as delegate to the Celebration of the One Hundred and Seventy-Fifth Anniversary of Rutgers University.

SCIENTIFIC PROCEEDINGS

THE COMPLEMENT-FIXATION TEST IN THE DIAGNOSIS OF SOME TYPES OF HUMAN ENCEPHALITIS. J. Casals and R. Palacios (by invitation), New York, N. Y.

Abstract. Fresh sera from 13 patients with encephalitis, previously diagnosed on the basis of neutralization tests, have been examined for specific complement-fixing antibodies with the following results: Specific fixation was obtained with sera drawn from 2 persons 8 years after an attack of louping-ill; from 5, 2½ years after an attack of Eastern equine encephalomyelitis, and from 2, 2½ years after Western equine encephalomyelitis; from 2, 4 to 6 months following St. Louis encephalitis, and from 2, 2 to 3 weeks after lymphocytic choriomeningitis. No fixation was obtained with sera drawn from 4 patients 4 to 8 years after an attack of St. Louis encephalitis; from 1, 5 years after lymphocytic choriomeningitis; from 2, with rabies, nor from 5 with fresh, undiagnosed, clinical encephalitis.

Discussion

(Dr. Leslie T. Webster, New York, N. Y.) The practical implication of this work is the development of a method for clinical diagnosis of the several virus encephalitides. Hitherto, the clinician has depended upon the neutralization test, a somewhat laborious procedure which cannot readily be carried out in all hospital laboratories but only in those in which the various viruses are maintained and tests are being run routinely. Dr. Casals' complement-fixation test is simple and practical. It remains to be seen how soon after onset of disease complement-fixing antibodies appear, in what proportion of cases, and how long they persist.

(Dr. S. Bayne-Jones, New Haven, Conn.) Dr. Casals gave the incubation temperature for rabbit, mice and guinea pig sera. Did you speak of the human serum incubation temperature?

(Dr. Casals) No; the human sera are usually incubated at 60° C. except in two instances. Wassermann-positive sera give a nonspecific reaction similar to the rabbit sera and must be incubated at 65° C. Sera from a few patients with encephalitis give a nonspecific reaction at 60° C. but on heating the serum at 65° C. the nonspecificity disappears.

THE CORRELATION BETWEEN ANATOMICAL CHANGES AND THE ALLERGIC STATE IN TUBERCULOUS GUINEA PIGS. C. E. Woodruff, H. S. Willis and (by invitation) Ruby G. Kelly, Northville, Mich.

Abstract. Guinea pigs were infected with 0.1 mg. of virulent tubercle bacilli. From the data obtained by testing these pigs at intervals of 2 weeks, with varying concentrations of O.T., skin sensitivity curves have been constructed. The healthiest animals reach a plateau of maximum skin sensitivity about 2 months after infection. At this time they give a definite reaction to 0.01 mg. O.T. Some animals fail to reach this plateau or, after reaching it, show a rapid decline in the level of skin sensitivity. The animals whose skin sensitivity falls below the 1 mg. reacting level at autopsy invariably show lungs filled with areas of tuberculous pneumonia in which may be demonstrated

innumerable acid-fast bacilli. Animals which die or are sacrificed when their skin sensitivity is at the 0.01 mg. level show only proliferative tubercles in the lungs. In these tubercles acid-fast bacilli are demonstrated with difficulty.

THE STRUCTURE OF BACTERIA AS SHOWN BY THE ELECTRON MICROSCOPE. Stuart Mudd and (by invitation) T. F. Anderson, K. Polevitsky and H. E. Morton, Philadelphia, Pa.

Abstract. Micrographs of bacteria made with the RCA electron microscope have shown very definite differentiation between a solid outer cell wall and an inner fluid, or potentially fluid, protoplasm. In chains of *Streptococcus pyogenes* and of aerobic spore-bearing bacilli the continuity of the chain has been shown to be due essentially to the continuity of the outer cell walls. Division in a strain of *Str. pyogenes* appears to be accomplished by pinching off the cell wall and its contained protoplasm. In species of the genus *Bacillus,* intercellular plates, as described by Knaysi and others, may be seen. Disruption of streptococci and bacilli by sonic vibration permits escape of the inner protoplasm, leaving the cell walls as "ghosts."

Cells of *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae* have been relatively transparent to the electron beam. In tubercle bacilli opaque granular bodies of various sizes may be seen in the inner protoplasm. In diphtheria bacilli grown on blood agar, very striking polar granules are found. In diphtheria bacilli grown on potassium tellurite medium, needle-shaped crystals of metallic tellurium are clearly visible within the cell protoplasm. Shaking such bacterial cells with bromine water dissolves the crystals, a behavior which is to be expected with tellurium metal.

Discussion

(Dr. Alex B. Ragins, Chicago, Ill.) I should like to ask Dr. Mudd whether bacteria of the Gram-positive series become Gram-negative when protoplasmic regression is demonstrated.

(Dr. Mudd) When the bacilli are fragmented in the sonic vibrator, the disintegrated or injured cells are Gram-negative. The uninjured cells remain Gram-positive.

(Dr. M. H. Soule, Ann Arbor, Mich.) How do you harmonize the opaqueness of *Bacillus megatherium* in this type of microscopy with the seeming transparency of this form under the dark field?

(Dr. Mudd) I have not time to go into the optics of this, but conditions are entirely different. Light goes through glass or quartz without interruption. Electrons are stopped by anything, even by air; all of this electron microscopy has to be done in a high vacuum. The lightness and darkness in an electron picture, as in an X-ray picture, are determined by the density and thickness of the object.

THE ELECTRON MICROGRAPHY OF PURIFIED VIRUSES. W. M. Stanley and T. F. Anderson (by invitation), Princeton and Camden, N. J.

Abstract. Viruses range in size from about 300 mμ to about 10 mμ. There has been considerable difficulty in their microscopy, since the limit of resolution for visual light is about 250 mμ. However, the recent development of an electron microscope with a resolving power extending down to about

5 mμ has made it possible to make micrographs of these small infectious agents. Micrographs of several purified viruses and of mixtures of viruses with homologous and heterologous antisera were shown.

Discussion

(Dr. Paul R. Cannon, Chicago, Ill.) I should like to ask Dr. Stanley if he were able to tell whether the antibody was dispersed evenly around the virus molecule or whether it was attached irregularly. The purpose of my question is to learn whether this method offers any additional information as to whether the antigen-antibody reaction is a physical adsorption or a chemical union to polar groups.

(Dr. Stanley) We can get some information, because the diameter of the molecule of the tobacco mosaic virus is increased from its normal value of about 15 mμ to a value of about 60 mμ following reaction with its antiserum. Since the molecule is a long rod about 280 mμ in length, it would appear that the entire molecule is covered and that the reaction is not localized at one single spot on the virus molecule. Since the size of the usual rabbit antibody molecule is about 3.7 mμ in diameter and about 27 mμ in length, it is obvious that the ends rather than the sides of the antibody molecules must become attached to the virus molecules in order to account for the observed increase in the diameter of the virus molecule.

You asked whether this is a physical adsorption or a chemical union. The fact that we observe no reaction between anti-bushy stunt virus serum and tobacco mosaic virus, or between anti-tobacco mosaic virus serum and bushy stunt virus indicates a remarkable degree of specificity. I think that this specificity must be similar to that which obtains in any ordinary chemical reaction.

(Dr. Stuart Mudd, Philadelphia, Pa.) There is one observation I should like to make regarding the remarkable agreement between the conclusions that have been derived from indirect measurements on the size and shape of virus particles by the various physical-chemical methods used, the conclusions which the immuno-chemists have derived from the reactions of antigen and antibody, and the results obtained by electron microscopy. The electron pictures confirm the conclusions from indirect methods to a remarkable degree.

(Dr. Thomas Francis, Jr., New York, N. Y.) May I ask whether the evidence is sufficiently clear so that you can say that the agglutination is entirely specific and that there is no foreign particle in these aggregations?

(Dr. Stanley) As you could see from the slides, the molecules of tobacco mosaic virus are rods about 15 mμ by 280 mμ, whereas the particles of bushy stunt virus are spheres about 26 mμ in diameter. The distinctive shapes and sizes of these two viruses, plus the fact that their rabbit antisera were available, provided the incentive for these studies. Although we are not immunochemists, it was obvious that these materials afforded a unique opportunity to study the antigen-antibody reaction under the electron microscope. The micrographs show that in a mixture of the two viruses with anti-bushy stunt virus serum, the tobacco mosaic virus molecules are unaffected and do not appear to be occluded in the precipitate formed as a result of the reaction between bushy stunt virus and its antiserum. The proof is rather good, for if tobacco mosaic virus were present in this precipitate it would be possible

to identify it because of its distinctive shape. I think it would be possible for a particle of bushy stunt virus to become occluded mechanically in a precipitate formed by tobacco mosaic virus and its antiserum, and we would not see it. In this case, there might be a small amount of mechanical occlusion, but certainly the great mass of material reacts specifically.

THE TREATMENT OF EXPERIMENTAL TUBERCULOSIS WITH PROMIN (SODIUM SALT OF P.P′ DIAMINO-DIPHENYL-SULPFONE-N,N′-DEXTROSE SULFONATE). William H. Feldman and (by invitation) H. C. Hinshaw and H. E. Moses, Rochester, Minn.

Abstract. Continuing a study previously reported on the effect of promin on experimental tuberculosis, 80 guinea pigs were inoculated with human tubercle bacilli (strain H37RV). Sixty-eight animals were treated with promin and 12 were kept as controls. Chemotherapy was started before infection in 20 animals, and in 48 animals treatment was withheld for varying periods up to 6 weeks after infection with tubercle bacilli. The experiment was terminated after 191 days. Results were as follows: Extensive and progressive tuberculosis in the controls and slight or no gross signs of tuberculosis in the treated animals. In only 3 of the treated animals was tuberculosis recognized grossly in the spleen. In the others that were treated with promin, visceral tuberculosis was apparently absent. The results suggest that under the conditions of the experiment promin proved to be an agent of considerable effectiveness in successfully combating experimental tuberculosis in guinea pigs.

Discussion

(Dr. Charles E. Woodruff, Northville, Mich.) I am very much interested in this paper. We attempted to treat two groups of guinea pigs, of 10 each, infected with tubercle bacilli, with promin given subcutaneously and noticed no beneficial effects. As a matter of fact, the mortality in pigs which received daily subcutaneous injections of promin was rather high. It is of considerable interest that in these animals which were fed promin the drug apparently had a beneficial effect.

(Dr. Feldman) We have had no experience with the drug given subcutaneously.

(Dr. M. M. Steinbach, New York, N. Y., by invitation) I would like to ask why the drug was given by mouth, as there are some indications that it is toxic by that method. Is that true for man or guinea pig?

(Dr. Feldman) We gave the drug by mouth because we were too lazy to give it four times a day parenterally.

(Dr. Steinbach) I think Dr. Sharpe, Medical Director of Parke, Davis and Co., indicated in a private communication that it was rendered toxic when given by mouth.

(Dr. Feldman) This compound is not pleasant to take when you attempt to give it in undiluted form. The taste simulates a mixture of onion and asafetida. When mixed with the feed we added karo syrup which veiled the taste effectively, and the animals not only ate it well, but many of them doubled their weight.

(Dr. Steinbach) We are using it in tuberculous guinea pigs, giving it subcutaneously three times a day, and I admit it is a nuisance to inject the

animals every 8 hours. In some animals we are observing hemorrhages at the site of injection.

(Dr. Raymond H. Goodale, Worcester, Mass.) May I ask the doses given the guinea pigs?

(Dr. Feldman) We kept the dose at 300 mg. per animal for each 24 hours

(Dr. Goodale) What was the total dosage given the animals?

(Dr. Feldman) My mathematics is not sufficiently good for an immediate answer.

A Study of Latent Lesions of Coccidioidomycosis Correlated with Coccidioidin Skin Tests. E. M. Butt and (by invitation) Arthur Hoffman, Los Angeles, Calif.

Abstract. Among 431 adult males on the medical service of the Santa Fe Coast Lines Hospital, a positive coccidioidin skin test was obtained in 18.7 per cent. These patients were from various cities and towns of California, Arizona and New Mexico. Fifty per cent of the patients from the San Joaquin Valley reacted positively to the coccidioidin skin test, whereas for the San Francisco area the figure was 23 per cent. The percentage of positive reactors from Arizona and New Mexico was 11.9 and 10.5 per cent respectively.

The lungs of patients coming to autopsy were removed intact and examined by X-ray for fibrotic and calcified lesions. Such lesions were excised, cultured and in some instances injected into guinea pigs. Eleven of the 431 cases have been autopsied. Four of the 11 had positive coccidioidin skin tests. No lesions suggestive of an arrested or healed coccidioidal granuloma were found in the negative reactors. Five of the 7 negative cases had evidences of a healed tuberculosis. Animal inoculations and cultures of this material were negative.

A positive culture of *Coccidioides immitis* was obtained from 1 of the 4 positive cases. Histologically the spherules of the fungus coccidioides were found in 3 of the 4 positive reactors.

The pulmonary lesions consisted of small encapsulated areas of caseation located in the parenchyma of the lungs. Histologically the capsules were noted to be composed of dense hyalinized fibrous tissue in which there were varying amounts of calcium. The centers were caseous. In all 4 cases arrested or healed lesions were found in the peribronchial lymph nodes. Spherules were demonstrated in the peribronchial lesions in 2 of the 4 cases.

Discussion

(Dr. Max Pinner, Bedford Hills, N. Y.) I should like to ask whether tuberculin tests were made in any of the positive cases.

(Dr. Butt) We have started another series of 500 cases that are being skin-tested with coccidioidin. Tuberculin skin tests are being performed on the positive reactors to coccidioidin. So far there has been no correlation between the positive reactors to coccidioidin and the tuberculin skin tests. About one half of the cases reacting positively to coccidioidin react positively to the tuberculin test. In the series of 431 cases reported, no tuberculin skin tests were performed.

(Dr. Esmond R. Long, Philadelphia, Pa.) This is a very important finding because it makes it necessary for us to revise our interpretation of X-ray

films in areas where this disease may be expected. I should like to ask two questions: first, does Dr. Butt think there might be other mycotic lesions which have a similar calcification? I ask that because there is one very large area in this country, the region in the east-central part of the United States, in which calcific lesions are very common in people who do not react to tuberculin. A certain percentage of these people, but not all, appear to react to coccidioidin; as far as I know there has been no direct association established between reactions in that area and such calcific lesions as you have shown. The second question I want to ask was touched upon, and that is whether a real primary complex developed?

(Dr. Butt) In answer to your second question concerning the relationship of the primary coccidioidomycotic lesion to the primary complex of tuberculosis, it is my impression that they are identical. The primary lesion of coccidioidomycosis is in the periphery of the lung with extension to the peribronchial lymph nodes. The first case presented today is an excellent example. A healed lesion was found in the upper lobe of the right lung, indistinguishable grossly from a primary tuberculous infection. Also healed lesions and the spherules of *C. immitis* were found in the peribronchial lymph nodes.

We have considered the possibility of other mycotic infections as the cause of calcified lesions in the lungs and peribronchial lymph nodes. So far, however, we have not demonstrated other causes for such healed or arrested pulmonary lesions. When one considers that in the past few years we have had almost as many autopsied cases of torulosis as we have had of the disseminated form of coccidioidal granuloma, the question arises whether or not some of these healed lesions are the result of a torula infection.

(Dr. William H. Feldman, Rochester, Minn.) Have you made studies of the histopathology of the local skin reaction in the positive cases?

(Dr. Butt) These are living patients, and usually the lesion has disappeared by the time of autopsy.

(Dr. Feldman) I had in mind material obtained by biopsy.

(Dr. Butt) No, we have not examined biopsy specimens.

SOME PATHOLOGICAL ASPECTS OF HUMAN MALARIA. Paul R. Cannon, Chicago, Ill.

Abstract. Material was presented contrasting the pathologic effects in 2 patients who died during active malarial infection. In 1 patient the infection was of a benign tertian type, whereas in the other it was malignant estivo-autumnal. Both patients were in middle age. Both were untreated so far as malaria was concerned and the duration of active symptoms was approximately the same, being 9 days in the case of the malignant malaria, and 11 in the benign infection. Histopathologic examination revealed the following:

In the benign infection malarial parasites had practically disappeared from the blood stream and only residual pigment could be seen in the spleen, liver and, to a lesser degree, in the bone marrow. No evidences of phagocytosis could be seen in any other organ examined, indicating that in the early stages of the benign infection the parasites were eliminated practically exclusively by these three organs. The lymphoid structures of the spleen were entirely normal, and there was no evidence of toxic effect upon the hepatic cells. In the patient dying from malignant malaria, on the other hand, the parasites were particularly numerous in certain regions. There was an enor-

mous concentration in the red pulp of the spleen, with innumerable parasitized erythrocytes being crowded together with no intermingling of leukocytes or of nonparasitized erythrocytes. Many capillaries in the myocardium, intestinal mucosa, pancreas and elsewhere were practically occluded by rows of parasitized erythrocytes without any intermingling of leukocytes or nonparasitized erythrocytes. Masses consisting entirely of parasitized erythrocytes were found in the lumen of pulmonary veins. Capillaries in the intestinal mucosa contained rows of parasitized erythrocytes with adjoining vessels filled with nonparasitized erythrocytes. The malpighian corpuscles in the spleen were markedly depleted and contained primitive undifferentiated mononuclear cells. There were marked fatty changes in the hepatic cells.

The pathologic changes in these 2 cases indicate that in the benign infection the parasites were easily disposed of by the main components of the reticulo-endothelial system, whereas in the malignant infection the parasites continued to develop despite the marked stimulation of lymphoid structures in the spleen and the efforts of the liver and spleen to remove them. The unequal distribution of clumps of parasitized erythrocytes, particularly as seen in the pulmonary veins and in the capillaries, suggests that this clumping may be the result of a specific antibody action. It is known that in malignant malaria of the monkey due to infection with *Plasmodium knowlesi*, agglutinins have been demonstrated which cause a clumping of parasitized red cells. The possibility that this also occurs in malignant human malaria is suggested by the fact that the masses of parasitized erythrocytes do not contain within them leukocytes or nonparasitized erythrocytes. It would seem that this latter condition would be present if the cause of the clumping is due, as has been usually assumed, to injury to the surface of the parasitized erythrocytes, with a resulting nonspecifically increased stickiness of the erythrocytic surface. Whether this tendency to embolic blockage of capillaries is due to specific or nonspecific factors, it would seem that the way to prevent this important complication of malignant malaria would be earlier and more effective drug therapy in order to keep to a minimum the relative numbers of parasitized erythrocytes.

Discussion

(Dr. George H. Whipple, Rochester, N. Y.) These pictures bring back the familiar appearances that we used to see constantly in the Canal Zone in 1907 and 1908. There, too, we were fortunate in being able to obtain material within an hour or two after death. The cases I remember particularly were those that came in in coma from the line, and were autopsied very shortly after death, and in almost all such cases a malignant type of malaria was found in which the capillaries of the brain cortex would be stuffed with these parasitized cells. The feeling of the physicians was that in this type of malaria with coma, nothing could be done by the largest doses of quinine intravenously, whereas if we could get them when they were beginning to show stupor they could be saved by heroic doses of quinine by vein.

(Dr. Stuart Mudd, Philadelphia, Pa.) I wonder whether it is possible to exclude the possibility that the change in the red blood cell might be nonspecific; it is known that injured red cells are easily taken out of the circulation by the spleen, so that there must be some nonspecific change in the red cell surface as a result of injury.

(Dr. Jacob Furth, New York, N. Y.) In regard to the specificity of this phenomenon described by Dr. Cannon it is noteworthy that a similar phenomenon occurs in leukemia, namely, leukostasis. The accumulation of primitive blood cells in the capillaries is likewise "selective" in leukemia, and there are often, side by side, capillaries distended with leukemic cells and capillaries filled with normal blood. It is unlikely that antibody formation is responsible for this phenomenon.

(Dr. Henry E. Meleney, Nashville, Tenn.) It is very well known, of course, that in estivo-autumnal malaria, during the second half of the 48-hour cycle, the parasitized erythrocytes usually are much fewer in the peripheral circulation, and only ring forms and crescents are usually found. It is generally believed that this is due to the stickiness of the parasitized red cells in the capillaries. This happens even in the first cycle of the estivo-autumnal infection, and would, therefore, not be due to a specific agglutinin. Also, in some cases which I have seen, the larger venules in the brain and other organs have shown individual parasitized red cells forming a ring adjacent to the endothelium with nonparasitized red cells in the center of the lumen. This did not appear in the vessels which Dr. Cannon showed, but it certainly does in some cases.

(Dr. Cannon) With regard to Dr. Mudd's question as to whether this reaction within the capillaries is specific or nonspecific, I should say that the simplest explanation and the one that has usually been followed is that the reaction is nonspecific and due to injury of the parasitized cell. It seems to me, however, that if the reaction is nonspecific there should be nonparasitized cells entangled in the clumps of parasitized cells together with fibrin and leukocytes. The fact, however, that the emboli consist entirely of parasitized erythrocytes points quite clearly, it seems to me, to the idea that this clumping is due to a specific mechanism. Complete proof is impossible because we must depend solely on morphologic evidence. Inferential evidence, however, substantiates the idea of a specific effect in that in monkeys infected with *P. knowlesi* agglutinins have been demonstrated (Eaton and Coggeshall) and actual agglutination in the circulating blood has been shown (Knisely). The idea of a specific agglutination does not eliminate the possibility that nonspecific factors may also play a part, as Dr. Furth has mentioned in connection with leukemia.

A Nonvirulent Irradiated Rabies Vaccine. Leslie T. Webster and (by invitation) J. Casals, New York, N. Y.

Abstract. The supernatant of a 1 to 5 per cent dog brain virus suspension exposed to ultraviolet light for 20 to 30 minutes becomes nonvirulent for mice and yet 0.1 cc. of this preparation immunizes them against a subsequent intramuscular injection of street virus. Thirty cc. quantities of this irradiated vaccine immunize beagle dogs.

The Histopathology of Histoplasmosis in Man. Robert J. Parsons, Ann Arbor, Mich.

Abstract. The presentation consists of the demonstration of the gross and microscopic characteristics of the lesions found in the 4 cases of histoplasmosis which have been seen at the University Hospital. Complete autopsies were done in 3 of these cases and *Histoplasma capsulatum* was grown from the tissues of 2 of the cases.

The lesions demonstrated from 1 or more of our cases are: ulcerative granulomatous lesions in the skin, pharynx, tonsils, hypopharynx and larynx; chronic ulcers of the naris, ileum and colon; extensive confluent chronic lobular pneumonia due to the fungus; miliary tubercle-like lesions in the lungs of 2 cases (tubercle bacilli could not be stained); severe subacute granulomatous portal cirrhosis of the liver. Almost complete caseation necrosis of both adrenals was seen in 2 of the cases, and these were the cases in which miliary lesions were found in the lung. Parasitized large mononuclear phagocytes were seen in the bone marrow, kidney, spleen, thyroid and many lymph nodes. In 1 case the organisms were found in polymorphonuclear cells in the circulating blood.

The yeastlike form of *Histoplasma capsulatum*, or an organism morphologically indistinguishable from it, was found in all of the above-mentioned lesions with the exception of the miliary tubercles in the lungs of 1 of the cases.

Discussion

(Dr. Henry E. Meleney, Nashville, Tenn.) I think Dr. Parsons' paper is important in calling attention to this interesting fungous disease. When the previous paper on Coccidioides was being discussed, the question was raised about calcification in the old lesions of that infection. I have seen slides from many of the cases that have been reported as histoplasmosis and have never seen calcification in any of them. The association of this disease with tuberculosis is interesting. I have recently reported 2 cases of histoplasmosis associated with tuberculosis which occurred at the Vanderbilt University Hospital in Nashville. This paper is in press in the American Review of Tuberculosis. In both of these cases the *Histoplasma* organisms were limited to the lungs. In one a very extensive infection was present, a pneumonia such as was described by Dr. Parsons. In the other case there were cavities in the apices of both lungs, but only in the walls of these cavities were a few mononuclear cells found containing *Histoplasma*. In the first case there was miliary tuberculosis, with acid-fast bacilli found in the tubercles in the liver, spleen and lungs, so that I think the two diseases are often associated. On the other hand, there have been several cases in which tubercle-like lesions have been described which do not look exactly like tubercles, and yet neither *Histoplasma* nor acid-fast bacilli have been found in them. In one of our cases there were in the liver, associated with the necrotic lesions, some granules in large mononuclear phagocytes which looked as though they might be degenerated parasites. In a recent case from the Los Angeles General Hospital, which has not yet been published, the adrenals were involved and caseous, just as they were in the case reported by Dr. Parsons, and there were tubercle-like lesions throughout other organs without tubercle bacilli and also without *Histoplasma* organisms, although a few suspicious organisms were found. I should like to say a word of warning in connection with the reporting of this disease. I have recently had referred to me 3 cases of supposed histoplasmosis in which the lesions consisted of isolated nonulcerated skin nodules. Dr. Dawson of the Department of Pathology at Vanderbilt University agreed with me that they were not histoplasmosis, and he believed that at least 2 of the cases were adenomas of the sweat glands. In the large cells there were granules which looked a little like *Histoplasma*, but they had no capsule and no definite morphology.

(Dr. A. M. Pappenheimer, New York, N. Y.) I should like to ask whether the massive necrosis of the adrenal led to symptoms of Addison's disease.

(Dr. Otto Saphir, Chicago, Ill.) Is there any histological or morphological characteristic that distinguishes this fungous disease from other fungous granulomas?

(Dr. Parsons) In reply to the question as to symptoms of Addison's disease, the man whose picture I showed had a very low blood pressure, 82/60; there was no pigmentation, but we were not smart enough to study him for Addison's disease, in spite of the fact that it was the second case of complete caseation of the adrenals that we had seen in our 4 cases of histoplasmosis.

The morphological characteristics are very distinctive. The organism itself varies from 1 to 5 μ in diameter, or 6 μ, if you include the capsule. For the most part the large mononuclear cells, or histiocytes, or whatever one wants to call the large mononuclear phagocytes, are invaded. There is, so far as I know, no other fungous infection which produces this picture, with one exception, and that is the disease, Neapolitan farcy, seen in horses in the Mediterranean Basin. However, the fungus causing farcy is culturally very different from *Histoplasma capsulatum*. I did not mention that we had cultured *Histoplasma capsulatum* from 2 of our 4 cases. I am quite convinced that there may be some other organisms that have not been identified which might resemble this, but so far as I know the morphological features are quite characteristic. Final identification of the causative organism must be made on the basis of cultural studies.

THE HISTOPATHOLOGY OF EXPERIMENTAL AND SPONTANEOUS ENCEPHALOMYELITIS OF MICE (THEILER'S DISEASE). Peter K. Olitsky and (by invitation) R. Walter Schlesinger, New York, N.Y.

Abstract. Further evidence of the similarity between human poliomyelitis and spontaneous encephalomyelitis of albino mice (Theiler's disease) was obtained from an examination of more than 5,000 semi-serial sections from 30 brains and cords deriving from mice spontaneously attacked by the disease or experimentally infected with Theiler's virus, and by comparing them with sections obtained from albino mice infected with the Lansing strain of human poliomyelitis.

Lesions of two basic types are seen: (1) Mesodermal-glial; consisting of perivascular microglia and round cell infiltrations, perivascular gliosis, diffuse and focal gliosis and endothelial swelling and proliferation in smaller blood vessels; and (2) neuronal. Neuronal changes ranged from early stages of tigrolysis to necrosis, and finally to complete disappearance with resulting vacuolization of the stroma. Neuronophagia was less extensive than in human and experimental monkey poliomyelitis, and showed predominance of microglial elements rather than of polymorphonuclear leukocytes. Intranuclear inclusion bodies, type B, were seen in early stages and were of the same type as those found in human and experimental poliomyelitis of the monkey. The distribution of lesions in Theiler's disease revealed no significant differences, whether the infection had been spontaneous or induced by intracerebral, intranasal, intralingual or intraperitoneal injection of virus. (The latter peripheral routes of inoculation were employed successfully in about 10 per cent of mice 2 weeks old.) In contrast to the positive findings in experimental poliomyelitis in monkeys, no lesions were found in the

olfactory bulbs of intranasally infected mice. Following intracerebral inoculation, the first lesions were observed around the site of injection, whence they spread mainly periventricularly. Invariably the rostral parts of the brain and the cortex showed a predominance of mesodermal-glial lesions, while in the substantia nigra and throughout pontine structures and the medulla, neuronal changes were prominent. The anterior horn of the cord showed both types but, in contrast to the findings in experimental poliomyelitis in monkeys, apparently normal neurons were always present to a considerable number. No characteristic differences could be found between the lesions in the central nervous system of mice caused by the Lansing poliomyelitis strain and those induced by Theiler's virus.

DESCRIPTION OF SPECIMENS OF PELLAGRA. Robert A. Moore, Tom D. Spies, Zola Cooper (by invitation) and Harry Goldblatt, St. Louis, Mo., Birmingham, Ala., Cleveland, O.

Abstract. Two biopsies of the skin were examined from each of 15 patients who had pellagra and who had not received treatment. One biopsy was taken from an area of skin showing clinical evidence of pellagrous change; the other was taken from an area which was apparently unaffected. The skin from the unaffected areas showed little deviation from the normal. The epidermis, however, in all of the cases showed a slight hyperkeratosis, and in 9 of the 15 cases, focal areas of atrophy. The dermis was slightly edematous, and in 4 cases a mild infiltrate of lymphocytes was observed in the upper third of the corium. In 4 cases the sebaceous glands appeared atrophic. The biopsies taken from pellagrous lesions uniformly showed marked evidence of pathologic change. The epidermis in all cases was markedly hyperkeratotic, with patchy areas of parakeratosis and thickening of the glandular layer in 4 cases. There was in all cases also a marked acanthosis with lengthening and thinning of the rete pegs, and in some cases intracellular edema. In 3, foci of atrophy were present in the otherwise acanthotic epidermis. Vesicles which had been infiltrated with lymphocytes and polymorphonuclear leukocytes were found at the dermal-epidermal junction in 4 cases. In isolated cases there was an increase in pigment in the basal layer, and pigment could be found also in the spinous layer and even between the keratinized layers of the stratum corneum.

The dermis was in all cases edematous, and there was marked dilatation of the peripheral blood vessels. A moderate lymphocytic infiltrate was present in the upper third of the corium. In isolated cases the collagen fibers in the deep corium showed evidence of hyalinization and mucoid degeneration with necrosis of individual fibers. In 8 cases there was an apparent absence of sebaceous glands in the biopsies studied. This was thought noteworthy in view of the fact that the lesions were in an active stage and had not yet become atrophic.

Discussion

(Dr. Stuart W. Lippincott, Bethesda, Md.) I want to ask Dr. Moore whether he feels it is possible to separate these histological lesions in the skin in pellagra from those in patients who probably have an associated deficiency of riboflavin or pantothenic acid.

(Dr. Moore) I think this has been taken care of by Dr. Spies's observations that these areas of skin in every case responded to nicotinic acid

therapy. I do not believe that the lesions described represent the complex of the various deficiency components, but that they are specifically due to a deficiency of nicotinic acid.

VITAMIN A DEFICIENCY AND THE CENTRAL NERVOUS SYSTEM. S. B. Wolbach and (by invitation) O. A. Bessey, Boston, Mass.

Abstract. Injury to the nervous system in vitamin A deficiency occurs only if the deficiency is established in very young, actively growing animals. We have been unable to produce lesions of the nervous system in white rats by vitamin A deficiency established after 10 to 12 weeks of normal growth. If the deficiency is established sufficiently early in life, neurological lesions invariably develop. Our procedure has been such as to prevent any considerable storage of vitamin A after birth and to place the rats on a vitamin A-free diet when weaned at 21 days of age. Ataxia and paralysis appear at about 50 days of age which is before the growth rate, as measured by weight increase, is appreciably changed. Administration of carotene at 42 days of age prevents the development of nerve lesions even though the diet is so restricted in amount that the treated animals grow less rapidly than litter mate controls continued on the vitamin A-deficient diet.

The neurological lesions in dogs, guinea pigs and rats are due to a disproportionate growth of the nervous system and skeleton. Our studies have been made chiefly upon white rats and confirmed in dogs and guinea pigs. In white rats the growth differential established by the deficiency is manifested in the gross by (1) overcrowding of the cranial cavity shown by distortion of the brain, dislocation toward the foramen magnum and multiple herniations of cerebrum and cerebellum into the venous sinuses of the dura at sites of arachnoidal villi; (2) overcrowding of the spinal canal with distortion of the spinal cord and herniations of nerve roots into intervertebral foramina and into bodies of vertebrae; (3) resorption of bone of the cranium and bodies of vertebrae in consequence of pressure. Early lesions of brain and nerve roots of the kind described in (1) and (2) have been found in rats killed before it was possible to elicit signs of functional disturbance.

A complete elucidation of the problem presented by these facts has not been achieved. The fact that vitamin A deficiency retards growth of bone must be taken into account. However, the study of rats whose growth rate has been more severely retarded by other deficiencies has shown normal relations of nervous system to skeleton. Litter-mate control work to date indicates that in vitamin A deficiency the nervous system grows at the same rate as in normal animals until that stage is reached where secondary starvation effects become operative. There is good evidence for believing that in vitamin A deficiency skeletal growth is retarded before that of the soft tissues in general.

Variations in distribution of the effects of the disproportionate growth in different species indicate that the order of initiation and rate of development of centers of ossification must be considered and suggest the possibility that a specific growth factor in bone is affected in vitamin A deficiency.

Histological studies have confirmed the conclusion that the nervous lesions in young animals, due to vitamin A deficiency, are mechanical in origin and due to the described disproportionate growth.

THE DISTRIBUTION OF VITAMIN A IN OVARIES AND OVARIAN TUMORS. Hans Popper and Alex B. Ragins, Chicago, Ill.

Abstract. The fluorescence microscopic demonstration of vitamin A in tissues (Querner, Popper) was applied to the human ovary. One observes a dull green, very slowly fading fluorescence due to carotene, a bright green, quickly fading, and a fainter green, slowly fading fluorescence, both due to vitamin A and imparted by lipoids. In rat ovaries evidence exists for the specificity of this fluorescence for vitamin A; in the human ovary no other substance has as yet been found imparting a similar fluorescence. After the second postfetal month there appear fine droplets with vitamin A fluorescence in the stroma around the primordial follicles and in the maturing follicles a quickly fading fluorescence in the granulosa and a slowly fading one in the theca. The theca of the corpora atretica shows larger amounts of the fainter fluorescence.

After puberty the stroma around the primordial follicles is free of vitamin A fluorescence. In the granulosa of the graafian follicles fine droplets with vitamin A fluorescence are seen, whereas the theca cells contain the fainter green fluorescence. After ovulation the bright fluorescence of the granulosa imparted by fine droplets increases, especially in the stage of vascularization and maturity. A dull green carotene fluorescence appears in the cytoplasm of the cells. In involution a strong, but more slowly fading fluorescence is imparted by large droplets. Later on the vitamin A fluorescence disappears prior to the lipoids. The corpus luteum of pregnancy reveals vitamin A fluorescence in the theca cells, the granulosa showing only strong carotene fluorescence. The atretic follicles and the scattered theca cells impart a strong, slowly fading fluorescence. After fading, a brown fluorescence is visible which in older corpora atretica is seen immediately. The brown fluorescent material is a non-lipoid-soluble pigment, dark brown in visible light. After the climacterium no vitamin A fluorescence is visible. The physiological significance of the vitamin A fluorescence in the ovary is not established. The characteristic cyclic variations of the fluorescence suggest a relationship of the substance with vitamin A fluorescence to hormone production. The fluorescence may be considered as a morphological sign of hormonal activity.

Generally tumors impart vitamin A fluorescence when their mother tissue shows the fluorescence. Consequently granulosa cell tumors in places reveal the fluorescence of the luteinized granulosa cells, theca cell tumors that of the theca cells of the corpora atretica, xanthofibromas that of a cortical stroma with vitamin A fluorescence, arrhenoblastomas that of the Leydig cells, dysgerminomas that of the dysgerminoma of the testicle. The specific structures of fibromas, papillary cystomas, cystadenocarcinomas and fibroepitheliomas are free from fluorescence.

EFFECT OF PANCREATIC ACHYLIA ON VITAMIN K ABSORPTION AND PROTHROMBIN TIME. Edith E. Sproul and (by invitation) Elmer Key Sanders, New York, N.Y.

Abstract. Adult cats were deprived of external secretion of the pancreas: 7 were subjected to sectioning of the pancreatic ducts and separation of the head of the pancreas from the duodenum; 2 received a complete pancreatectomy, and in 6 about two thirds of the pancreas was removed, leaving the

tail completely detached from the intestine. Two control operations, in which the tissues were handled but not cut, were performed and there were 3 unoperated control cats.

Disturbance in absorption of fats was manifest in the immediate rise in total lipid content of the stools from a normal level of 2 to 15 per cent to five or six times this amount. There was considerable fluctuation of prothrombin times. During the preoperative period these values varied within a range about 10 per cent above or below the average normal level. Following section of the ducts, fluctuations were enlarged to 48 per cent below and 116 per cent above the average normal values. After subtotal resection of the pancreas there was a marked prolongation of the prothrombin times, reaching an average of almost 100 per cent above the normal at one point. Again there was fluctuation in the readings and a gradual tendency toward restoration of the normal state. Whole blood clotting times were moderately elevated, the highest being 19 minutes following subtotal pancreatectomy. Bleeding times showed slight alteration.

The livers of the experimental cats were frozen and dried *in vacuo* promptly after death. The vitamin K content of the pulverized liver was determined by the Ansbacher curative method of bio-assay. The amount of vitamin was reduced after duct resection and markedly lowered following pancreatectomy. It was suggested that the loss of pancreatic lipase diminished the digestion of fats and absorption of the fat-soluble vitamin K and that production of prothrombin by the liver was accordingly impaired.

A Vitamin B_1 Deficiency of Foxes Produced by Feeding Whole Fish. Robert G. Green, Minneapolis, Minn.

Abstract. A widespread disease in foxes and mink called Chastek paralysis and caused by the feeding of various kinds of fresh uncooked fish has been found to be a vitamin B_1 deficiency. The disease may be produced by adding 10 per cent ground whole carp to the ration, or by adding a mixture of heads, skin, tails, and fins and some scales. A ration containing the fillets of carp has not produced the disease in comparative tests. Chastek paralysis may be prevented by adding large amounts of thiamin to the fish ration. In its clinical and pathological manifestations, the disease is analogous to Wernicke's alcoholic encephalitis of man.

Pathological Changes in the Mouse Due to Pantothenic Acid Deficiency. Stuart W. Lippincott and (by invitation) Harold P. Morris, Bethesda, Md.

Abstract. Pantothenic acid was first described in 1933 by Williams and associates as a bios or growth factor for yeast. During the last year it has been synthesized and proven to be necessary in the nutrition of the chick and rat. Because of its importance as a nutritional factor, its possible rôle in tumor metabolism *in vivo* in spontaneous mammary carcinoma of the C_3H mouse was investigated. Before proceeding to these experiments it was necessary first to establish whether the mouse required pantothenic acid and what the pathologic changes were in the deficient state. This report deals with the latter two phases.

In this strain of mice removal of pantothenic acid results in lack of growth in young animals, marked loss of weight in adults and inability of either group to survive for more than 8 weeks. During the course of the deficiency

there is depilation and scaling of the epidermis about the nose and over the scapular region and flanks. The animals become sensitive to touch, squeak in a characteristic manner, develop an awkward, stilted gait and finally partial paralysis of the hind legs. Histologically there is minimal fatty degeneration in heart, liver and kidney, as well as an hyperkeratotic, atrophic, desquamative dermatosis and myelin degeneration in the sciatic nerves and spinal cord, chiefly in the posterior funiculus. The gross and histologic changes are multiple manifestations of the widespread disturbance in normal tissue metabolism which occurs in the deficiency state after complete removal of pantothenic acid from the diet.

A Quantitative Study of Reversible Stabilization of Polysaccharide in Dyed Cartilage. George M. Hass, New York, N.Y.

Abstract. If fresh frozen sections of human epiphyseal cartilage are extracted at pH 11 for 24 hours at 5° C., a large percentage of chondroitin sulfuric acid is removed. If the sections are stained with crystal violet prior to extraction under these conditions, the polysaccharide is retained in the tissue in quantities which are proportional to the amount of dye bound in the tissue. If the tissues are stained with the dye and if the dye is subsequently extracted with alcohol, polysaccharide is set free at pH 11, provided the initial concentration of the dye in the tissue is not too great. Therefore, under the specified conditions of extraction, stabilization of chondroitin sulfuric acid in cartilage is almost completely reversible if a proper choice of concentration of dye in the tissue is made.

Occurrence of Urinary Tract Calculi in Inbred Strain (C_3H) of Mice Treated with Estrogen: Further Observations. John R. Schenken, Edward L. Burns and (by invitation) William M. McCord, New Orleans, La.

Abstract. Studies of estrogen-injected strain C_3H mice under controlled conditions revealed a high incidence of urinary tract calculi, particularly in males, as compared with a control group of animals. The injections, for which crystalline α-estradiol benzoate in sesame oil (progynon B) was used, were made in varying doses and for varying periods of time. The highest incidence was observed in males which had been treated for from 8 to 20 weeks and which then lived from 4.82 to 8.92 months longer. The calculi were of the "bone earth" type. Pathologic changes in the genito-urinary tract similar to those reported by other investigators were also observed. Apparently both metabolic and inflammatory factors may be responsible for the high incidence of calculi in estrogen-treated animals. The available evidence seemed to indicate that the metabolic factor was associated with a derangement of calcium metabolism. Other evidence indicated that epithelial hyperplasia with desquamation and urinary tract infection also played a part in the formation of calculi, particularly in male animals.

Discussion

(Dr. Harold L. Stewart, Bethesda, Md.) I should like to ask whether this strain of C_3H mouse was obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine.

(Dr. Schenken) We obtained the original strain from Bar Harbor; the animals were bred by brother-to-sister matings in New Orleans.

(Dr. Stewart) How long had the stock been separated from the parent strain?

(Dr. Schenken) Since 1937.

EFFECTS OF LONG-CONTINUED INGESTION OF SODIUM PHOSPHATE UPON THE PARATHYROIDS, KIDNEYS AND BONES OF MATURE RATS. John A. Saxton, Jr., and (by invitation) Gordon H. Ellis, New York City and Ithaca, N.Y.

Abstract. Mature male rats were divided into two lots. One lot was fed an adequate diet containing 8 per cent of sodium metaphosphate. The other lot received a diet containing about the same quantity of sodium orthophosphate. These diets were continued for 7 months, or until the animals succumbed. X-ray examinations at intervals during life disclosed a gradual decalcification of bones and deposition of calcium in the kidneys. Urinary excretion of inorganic phosphorus was increased. Gross examination showed enlargement of the parathyroids from two to eight times that of the controls. The long bones appeared slightly thickened, but in some instances were more fragile than normal. Microscopic study showed hypertrophy and hyperplasia of parathyroid cells. Calcium deposits were present in the tubules of the kidneys, chiefly in the medulla. There were widespread metastatic calcium deposits in numerous organs. The long bones showed diffuse endosteal and periosteal new-bone formation with focal areas of osteoclasis and fibrosis. These observations suggest that some instances of human disease in which there are lesions of the osseous system and parathyroid hyperplasia may be a consequence of excessive ingestion of phosphates.

THE NEURO-INSULAR COMPLEX OF THE PANCREAS: ITS POSSIBLE RÔLE IN DIABETES. Louis C. Simard, Montreal, Canada.

Abstract. In 1925 Van Campenhout described in the pancreas of certain mammalian embryos a structure which he called the sympathico-insular complex. It consists in the intimate association of insular and nervous elements by migration into the nerves of cells arising from primitive pancreatic ducts. These complexes were considered to be a stage in the organogenesis of the pancreas as transitory structures which insured an ephemeral or embryonic function, but which were destined to disappear. The author endeavored to follow the fate of these complexes in the pancreas of the adult man (1937), and in several other adult mammals. The pancreatic glands on which this study is based were removed from man, pig, dog, cat, white porpoise, horse, ox, sheep, rabbit, guinea pig, raccoon, opossum and rat. In all these animals the complex, which should be called the neuro-insular complex, was found.

The structure of the complex is not uniform; rather it is characterized by a great variability as regards the proportion of epithelial and ganglion cells. The epithelial cells of the complex are insular cells. From the histophysiological point of view the neuro-insular complex of the pancreas might be considered as a typical neurocrine organ, being a part of the intrinsic nervous system of the gland. It might be an important organ in the autonomic regulation of insulinic secretion.

Total cross sections of the pancreases taken from persons who had died in diabetic coma were carefully examined by serial sections. In 1 case only two

complexes could be found, in another, only one, and in the last 3, none was found. And yet, in the 5 cases selected, the islands of Langerhans showed no degenerative or hyaline lesions: 2 of them, on the contrary, had, as is frequently found, numerous hypertrophied islands. If one remembers (1937) that the complexes are quite abundant in man (7 in a series of 300 μ thick) one cannot but be impressed by the considerable reduction of the complexes in these pancreases.

This research should be continued on many other glands of diabetic patients, as it might throw some light on the pathogenesis of diabetes.

THE PATHOLOGY OF SHIELDED ARC WELDING. Emmerich von Haam and (by invitation) J. J. Groom, Columbus, O.

Abstract. The health hazards of the welding occupation are still under dispute among physicians and public health officials. Thus far almost every disease encountered in welders has been linked by some authority with the welding occupation. Because of the uncertainty of most of these statements, based solely upon clinical observations, we studied experimentally the lesions produced by exposure to welding fumes and welding gases. Rabbits, guinea pigs, rats and mice were exposed daily to the concentrated fumes and gases produced by three commonly used coated rods. The concentration of fumes was in most experiments far above the concentration levels commonly encountered in industry.

At the end of our experiments we were impressed by the low toxicity of the fumes and gases produced by the coated rods examined by us. The mixture of welding gases, which included a minimal amount of carbon monoxide and a varying amount of the "feared nitrous fumes," produced with some exceptions only irritative lesions of the trachea and bronchi. Never did the blood of the experimental animals show any significant rise of carbon monoxide following exposure. The welding fumes, whose composition varied with the type of rod, produced after prolonged exposure a heavy iron pigmentation of the lungs, which did not incapacitate in any way the respiratory function of the lung, such as is encountered in the common toxic dust diseases. Silicosis and asbestosis were not encountered. Common respiratory infections typical for the various species of rodents were observed more frequently during the period of exposure than during the period of observation. No significant changes in the blood counts, in the endocrine glands, the central nervous system, or in any other organs were observed. The animals frequently became pregnant and delivered healthy litters.

From the above results we felt justified in concluding that the welding products from coated rods of average composition, in the concentration commonly encountered in industry, do not possess toxicity as attributed to them by the largely speculative medical literature. For the occurrence of acute respiratory lesions an individual susceptibility and the existence of a previous or concurrent bacterial infection seem more important than the composition and concentration of the welding products.

Discussion

(Dr. Harold L. Stewart, Bethesda, Md.) I should like to ask if there was observed any increase in pulmonary tumors in the treated as compared with the control mice.

(Dr. von Haam) Two adenomas of the bronchial glands were found.

(Dr. W. C. Hueper, New York, N.Y.) Did you examine the brains of these animals? The fumes contain an appreciable amount of manganese and manganese is known to be toxic to the central nervous system.

(Dr. von Haam) We observed no changes in the brains in any of the animals.

(Dr. Samuel R. Haythorn, Pittsburgh, Pa.) The deposits in the lungs are almost identical with those obtained in experiments with bituminous coal smoke, and, as Dr. von Haam stated, are not at all similar to silicosis.

CIRCUMSTANCES AND POSTMORTEM FINDINGS, ESPECIALLY SKIN LESIONS, IN ACCIDENTAL ELECTROCUTION. Milton Helpern and (by invitation) George Strassmann, New York, N.Y.

Abstract. This study is based on 144 cases of accidental electrocution which include the majority of such cases investigated by the Office of the Chief Medical Examiner from 1928 to 1938, and a number of cases observed abroad from 1924 to 1938. Of these 144 cases, 101 deaths were caused by high-tension currents of 600 v. or more, and 43 deaths by low-tension currents of 110 and 220 v., usually of alternating type. In the high-tension group there were 64 immediate deaths and 37 delayed deaths; of the latter, 23 persons died within 48 hours and 14 died in from 2 days to 2½ months after the accident. In the low-tension group there were 37 immediate deaths and 6 delayed deaths caused by ignition of clothing by burning insulation or short circuit sparks, rather than from the effect of electric shock. Necropsies were performed in 50 of the high-tension and in 29 of the low-tension cases. The circumstances were investigated and careful examinations made of the bodies in all of the 144 cases.

Twenty-six of the low-tension and 65 of the high-tension accidents occurred during work; 11 low-tension deaths in the home; 29 high-tension deaths as the result of trespassing and contact with high-tension wires and third rails, and 7 deaths from contact with fallen high-tension wires. In both low and high-tension groups, most victims were in the third and fourth decades of life, with a considerable number of children in the high-tension group. Of the victims, 135 were males. In 13 of the low-tension cases the victim cried out when the shock was received; in 24, the victim was found dead, the accident not having been witnessed.

Most of the accidents in both the low and high-tension groups occurred during the warm months of the year, with the peak incidence in July and August. The seasonal incidence was especially striking in the low-tension group, and is dependent upon conditions favorable for the passage of low-tension currents through the body. Hot, humid weather induces sweating, and the moist skin has a lessened resistance to the current. Moist soil and damp shoes and feet furnish excellent conditions for grounding. A considerable number of low-tension accidents occurred while the victim was washing out a boiler, standing in water holding an extension lamp in one wet hand and a hose in the other. There were several cases in which the victim touched an electric appliance or fixture while taking a bath.

The general pathologic findings are not characteristic. The necropsies in cases of immediate death revealed visceral congestion, pulmonary edema, fluid blood in the heart, at times petechial hemorrhages in the pleura, peri-

cardium and conjunctiva. A stomach full of recently ingested food was often found.

The specific lesions are the current marks (Strommarke of Jellinek) and burns at the sites of contact, usually on the hands or fingers. In the low-tension group of 37 immediate deaths, these current marks and burns were absent in 11 cases, or in slightly less than one third. They were reported in all the others, although some were questionable in the absence of a microscopic examination and were usually found at the site of entrance of the current. In all but 1 of the high-tension group, current marks or burns were present, usually at the site of entrance and exit. In most cases of electrocution by low and high-tension currents there were marks and burns either on the left upper extremity, the left side of the trunk, the left leg or both legs, or on any of these sites combined with the right upper extremity, the distribution indicating that the path of the current traversed the heart and tending to support the theory that death results from ventricular fibrillation rather than from respiratory paralysis.

The gross appearance of the current marks is not too characteristic and their detection requires a careful search. They may appear as yellowish, slightly depressed, punctate, circular, elliptical or linear marks. There may be blisters occurring singly or in a row. Prolonged application of the current may produce charring. The microscopic picture is often characteristic. There is vacuolization or "honeycombing" (Schridde) of the stratum corneum, a splitting of the epidermis with threadlike elongation of the cells and nuclei of the deeper layers of the epidermis, the altered cells having a distorted whorled arrangement, or the layers of the epidermis and corium may be compressed and fused with alteration of the staining characteristics of the corium which appears lilac-colored in preparations stained with hematoxylin and eosin. Distortion and shrinkage of the cells and nuclei may be found in the hair follicles and sweat glands. Vesiculation of the epidermis is found in some cases and also charring of the skin and subcutaneous tissues. Microscopically, the current marks are distinguishable from antemortem abrasions and postmortem artefacts such as occur from vigorous attempts at artificial respiration or careless handling of the body. Blistering and separation of the epidermis resulting from postmortem putrefaction occur between the epidermis and the corium; the electric current splits the layers of the epidermis so that these marks can be distinguished from and are recognizable in the presence of putrefactive changes. The current marks do not represent a vital reaction, but are produced by the heat generated by passage of the current through the resistant skin. The marks may be indistinguishable from those produced by the application of hot objects to the skin and they may be simulated in this way.

In high-tension electrocutions, in addition to the ordinary current marks, there usually are extensive electrical burns due to ignition and incineration of the tissues, and also third degree burns produced by burning clothes ignited by the current. In high-tension cases with extensive burns, the ordinary current marks may be difficult to find.

Immediate death in high-tension electrocution may result from the combination of electric shock, extensive burns and blunt-force injuries sustained when the body falls or is thrown from a height at the time the shock is received. Delayed deaths from high-tension shocks may result from a com-

bination of shock, toxemia and septic complications of the burns, or from the complications of associated blunt-force injuries. Electric shocks by low-tension currents either kill immediately or not at all, and are usually without sequelae.

Discussion

(Dr. Howard T. Karsner, Cleveland, O.) In respect to this most interesting paper, I have one minor technical question. Some papers in the literature make the dividing line between high and low voltages 1000 v. In this paper it is placed at 600 v. How is the dividing line determined?

(Dr. Helpern) The dividing line is quite arbitrary. Currents of 110 and 220 v., usually alternating, are utilized in the home and in industry, and are commonly referred to as low-tension currents. High-tension currents range from 600 v. into the thousands and are transmitted by underground cables, overhead high-tension wires and third rails. We have not encountered any current voltages between 220 and 600. High-tension shocks are always dangerous to life. Low-tension shocks are frequently sustained without injury, but under certain circumstances may be fatal.

COAGULATION TIME OF THE BLOOD AND MURAL VASCULAR LESIONS AS DETERMINANTS OF THROMBOSIS. R. Katzenstein (by invitation), M. C. Winternitz and (by invitation) E. Mylon, New Haven, Conn.

Abstract. The well known effects of tissue extracts on the clotting time of the blood and the production of thrombi is related to their content of thromboplastic substance. This varies considerably with different tissues. Lesions of the walls of the heart and blood vessels are not necessary for thrombosis. Moreover, such mural changes occur without superimposed thrombi if the stability of the blood from the standpoint of its coagulation is not advantageous. Location of thrombi after injection of tissue extracts indicates variation in the coagulation time of the blood in different parts of the vascular bed and suggests participation of different organs in effecting the balance of the coagulation time.

LIGATION OF THE PULMONARY ARTERY: ITS RELATION TO PULMONARY ATELECTASIS, PULMONARY CIRRHOSIS AND BRONCHIECTASIS. Joseph Tannenberg, New York City and Bedford Hills, N. Y.

Abstract. In experiments on rabbits the relationship of pulmonary atelectasis and bronchiectasis was studied in several series which are reported elsewhere with Pinner in greater detail. In these experiments the bronchus of one lung was obstructed from within, or from without by ligation at the bifurcation. In a parallel series pneumothorax was maintained on the side operated upon for the duration of the experiment (up to 7 months); in another series infectious material was deposited within the bronchus prior to ligation; in a final series the ligation was made so that only partial bronchial obstruction resulted. These results were obtained. 1. Pulmonary atelectasis without complications could be maintained for several months unless infections intervened. 2. The bronchi within the atelectatic lung were maximally constricted, in spite of the increased negative intrapleural pressure at the side of the atelectatic lung. 3. Shift of the mediastinal organs and of the over-inflated contralateral lung, and elevation of the diaphragm took up the thoracic space made vacant by the shrinkage of the atelectatic lung. 4.

Bronchiectases from cylindrical to most severe saccular forms occurred as a consequence of infections producing purulent bronchitis in the obstructed lungs. 5. Under such conditions *pneumothorax* on the obstructed side did not prevent the formation of bronchiectases. 6. Partial obstruction of the main bronchus led to emphysema, not to bronchiectasis, if infection was prevented.

Ligation of the pulmonary artery on one side in a series of rabbits which were kept alive for a period up to 5 to 6 months had these results: Not simple atelectasis, but hemorrhagic infiltrations and necroses of varying extent were the early changes. If the animals survived the first few days, organization of the hemorrhagic necroses and subsequent fibrotic shrinkage of the involved areas developed. This quite different condition, nevertheless, produced practically the same roentgenological picture as pulmonary atelectasis, the same dense X-ray shadow of the involved lung, and shift of the mediastinal organs and the contralateral lung to the affected side.

After ligation of the pulmonary artery shrinkage of the lung takes place while the atmospheric air has free access to the bronchial tree. In this respect the conditions resemble closely those which exist in man when bronchiectasis develops. The results obtained fully confirmed those obtained after bronchial obstruction. If there was no complication by infection, no bronchiectasis developed despite shrinkage of the lung to a fraction of its normal volume. When spontaneous infection of the bronchial tree intervened, or upon artificial infection with a bovine strain of tubercle bacilli, bronchiectases of various sizes occurred; in the latter case, however, only when tuberculous foci had established themselves in a bronchial wall causing destruction of the bronchial musculature.

The pleural space made vacant by the shrinkage of the lungs was filled by shift of the mediastinal organs, over-inflation of the contralateral lung, and over-inflation and emphysema of those parts of the isolateral lung which were preserved.

Discussion

(Dr. Max Pinner, Bedford Hills, N. Y.) I think there is one important clinical and roentgenologic conclusion to be deduced from these experiments. The roentgenologic criteria for the diagnosis of pulmonary atelectasis; namely, the ground-glass appearance of the lung, the shift of mediastinum and diaphragm, and so on, are taken in general as proving the presence of atelectasis. However, it is shown by these experiments that other pulmonary conditions may produce identical roentgenologic appearances. This is true for the lung which becomes shrunken and organized and completely fibrotic following ligation of the pulmonary artery. It is equally true for the lung which is shrunken but infected and contains large bronchiectases. As I said in the beginning, the current criteria for pulmonary atelectasis are not sufficiently differentiating for uncomplicated atelectasis, but apply to a number of other pulmonary diseases as well.

WEIGHT OF THE NORMAL HEART IN ADULT MALES. Paul D. Rosahn, New Haven, Conn.

Abstract. A group of 187 males 20 years of age and over, with no cardiovascular disease at autopsy, dying either from trauma or from an acute disease requiring not more than 2 weeks' hospitalization, was studied to determine the influence of age, height and body weight upon the weight of the heart.

The mean age for the group was 44.1 years, and the average heart weight was 356 gm. A slight but significant positive correlation of 0.2670 was found between age and heart weight. It was not possible to exclude entirely the influence of hypertension as a factor accounting for this significant correlation, but it appeared that if hypertension rather than age were the influential factor, it did not account for more than 7 per cent of the variability in heart weight. Body weight and heart weight were significantly correlated in a positive direction, and this was independent of age. Height did not appear to contribute materially to the variability in the weight of the heart, except in so far as height affected body weight. The multiple correlation between heart weight and body weight and age was 0.6287. This gave the lowest standard error of estimate of any of the calculated correlation coefficients. The corresponding multiple regression equation was: estimated heart weight in grams = age in years + 3 (body weight in kilograms) + 100. It was concluded that a diagnosis of cardiac hypertrophy was justified when the observed heart weight exceeded by more than 77 gm. the estimated heart weight derived by the use of this regression equation.

Discussion

(Dr. Otto Saphir, Chicago, Ill.) It is very interesting to observe the heart weights in this presentation, because it appears to show that the heart weight is distinctly greater than one would have expected from the figures published up to the present time. I would like to be informed in regard to the microscopic changes in some of the hearts. About 66 of the hearts were taken from patients who had died of acute causes, and these were presumably normal hearts. From my own experience, and from that of others, it seems quite clear that some of these hearts might show evidence of acute myocarditis, or at least of edema, and I think the edema can be seen in microscopic sections. If these 66 cases had not been included I would have liked the results much better, but this edema which might have been present in the myocardium might outbalance the results of the other cases.

(Dr. E. T. Bell, Minneapolis, Minn.) I am inclined to put much more stress on hypertension as explaining the age increase in the heart weight than Dr. Rosahn does. It is well established that about 40 per cent of males over 50 years of age have a blood pressure of 150/90 or higher, and if you take out all hypertensives most of that age increase disappears.

(Dr. Rosahn) As I said in the presentation, I did not personally view the microscopic preparations of the hearts that constituted this survey. However, as a routine in the Department of Pathology, sections are taken from the heart and studied in conference and if no abnormality is present, the heart is considered to be a normal heart. I can say none of the cases had acute myocarditis. There is a possibility—I do not know how probable it is—that some of them did show some degree of edema. I will be very happy in further presentations on this subject to take that suggestion into consideration and include only those cases in which no edema can be shown microscopically.

With regard to Dr. Bell's question of hypertension being the factor involved rather than age in the increase in heart weight, it is not possible, of course, to exclude hypertension as the primary factor. But should one ascribe to hypertension alone all of the variability which is the result of age, then

hypertension, in this series at least, did not contribute more than 7 per cent to the total variability in heart weight, and that is the maximum contribution of age alone. I do not believe that hypertension *per se* is the important factor involved. As a matter of fact, certain of these cases were included which showed some degree of atrophy.

THE RELATION OF THE "MYOCARDIAL RETICULOCYTE" TO THE ASCHOFF NODULE. Benjamin J. Clawson, Minneapolis, Minn.

Abstract. The "Anitschkow myocyte" or myocardial reticulocyte of Ehrlich and Lapan is a cell with a peculiar morphology, characterized by having an elongated, serrated chromatin bar within the nucleus with fibrillar extensions toward and to the nuclear membrane. It is found only in the heart and heart valves. This cell responds in rheumatic inflammation by proliferating and by taking on more cytoplasm which stains darkly. It is often the chief cell found in Aschoff nodules and appears always to be present in a greater or less degree. The nuclei in the giant cells in the nodules have the typical morphology. The response of this cell is not characteristic of rheumatic inflammation, for the cell is not found in rheumatic subcutaneous nodules and is found in nonspecific inflammation in the heart. The presence of this peculiar cell in rheumatic inflammation may help to explain the term "typical Aschoff nodule."

Discussion

(Dr. Paul Klemperer, New York, N. Y.) Dr. Ehrlich, working in our laboratory, has worked along the same line. I should not like to call the cell a "histiocyte"; it is a multipotent cell and we used to refer to it as the "mesenchymal cell" of the cardiac skeleton. I have seen, and I wonder whether Dr. Clawson agrees with me in this, a transition of this type of cell into fibroblasts. The cell is stimulated in general infections without any conspicuous lesion of the heart. It is more frequent in children than in adults.

(Dr. Clawson) I have seen a cell of this type apparently changing to a fibroblast.

THE PATHOGENESIS OF ARTERIAL ATROPHY. Alan R. Moritz, Boston, Mass.

Abstract. Varying degrees of local circulatory stasis were induced in segments of carotid and femoral arteries of dogs and the subsequent reactive vascular changes were found to be similar to those which occurred in the splenic arteries of infants and children following splenectomy. Three types of adaptive vascular change were observed as a result of diminished blood flow. In some arteries contraction unaccompanied by fixed tissue proliferation comprised the full extent of the reaction. In others intimal hyperplasia was superimposed upon the vascular contraction. There was evidence that intimal hyperplasia may occur without antecedent mural thrombosis. New elastic fibers were formed eventually in the hyperplastic intima. In still others the obstructed segments of vessels were occluded by thrombosis.

These experimental involutional changes, if they may be designated as such, are similar in some respects to those which occur in the intra-abdominal portions of the umbilical arteries of young infants, in the uterine arteries of young women during the postpartum period, in the cortical ovarian arteries of women during the catamenial period of life and in the intermediate and

small arteries of the atrophic kidneys of otherwise nonarteriosclerotic persons with chronic Bright's disease. Although it remains to be demonstrated whether or not obliterative renal arterial change may be caused by reduced blood flow through the kidneys, evidence has been presented that similar changes in other vessels are often adaptive and occur as a result of reduced circulation.

Discussion

(Dr. Joseph Tannenberg, Bedford Hills, N. Y.) I would like to ask Dr. Moritz if there were any changes in the nutritional vessels or vasa vasorum of the isolated arterial segment, when proliferation of the intima was observed?

(Dr. Moritz) The only fixed tissue changes seen in isolated segments were in those in which thrombosis occurred. Up to 43 days no intimal changes were noted in isolated segments which had been washed clean so that there was no thrombus.

THE PATHOLOGY OF ARTHRITIS DEFORMANS. S. A. Goldberg, Newark, N. J.

Abstract. In a study of joints from surgical and autopsy material, lesions have been encountered that appear to divide arthritis deformans into two main groups: those in which the lesions are possibly due to an infective agent and those in which the lesions are possibly due to trauma. This trauma may be extrinsic or due to the wear and tear of advancing age. There are cases of arthritis deformans in which there appears to be a combination of these two groups of lesions.

In the first group the synovial membrane is thickened by villous or papillary growths of fibrovascular tissue containing perivascular lymphocytic or plasma cell infiltration. The articular cartilage may be completely replaced by granulation tissue, the areas of remaining cartilage degenerated and covered by vascular tissue containing lymphocytic infiltration. These changes do not result in true ankylosis. Inability to move the joint is due to pain or to distortion of the articular surfaces. The subchondral bone is atrophied, possibly due to disuse. In the early stages there is a synovial pannus in the joint producing erosion of the articular cartilage by direct dissolution, suggestive of enzyme action.

In a study of early lesions of arthritides in young animals, (Equidae and Bovidae) it was seen that the earliest pannus formation is a thin vascular film emanating from the synovial membrane, from around the interosseous ligament, or from the subchondral marrow of a preëxisting articular erosion. The erosions may also be formed by penetration of the articular cartilage by fibrovascular tissue from the subchondral bone, as shown by Bauer. In the early lesions the cartilage at first shows a change in the staining reaction. Normally cartilage takes the alkaline stain and appears blue. In these lesions the cartilage takes the acid stain and appears pink. This indicates a change in the pH of the cartilage before the cartilage completely disappears. Eventually portions of the subchondral bone also become eroded and replaced by fibrovascular tissue. Ankylosis may follow these erosions or the vascular pannus, even without complete destruction of the opposing articular cartilages.

In the second group the articular cartilage is thickened by proliferation of cell nests that later undergo degeneration and take the form of fibrils per-

pendicular to the articular surface. The subchondral bone does not appear to be involved in this process. This is interpreted by Parker and Keiffer as an anatomical condition due to advancing age, and by Callender and others as a degenerative arthritis. In this series of cases, one of which was in a patient 5 years of age, the lesions were associated with definite trauma. In the group of older patients the lesions in the articular cartilage were possibly caused by circulatory disturbance produced by endarteritis obliterans of the anterior and posterior tibial arteries. All the patients complained of pain. This was probably due to stretching of the synovial membrane since nerve endings have not been demonstrated in articular cartilage or in subchondral bone. This condition is extremely common in man and animals as pointed out by Callender and Kelser.

THE PATHOLOGY OF THE JOINT LESIONS IN PATIENTS WITH PSORIASIS AND CHRONIC ARTHRITIS. Granville A. Bennett and (by invitation) J. Wallace Zeller and Walter Bauer, Boston, Mass.

Abstract. Included among 31 autopsied, and approximately 100 surgically treated, cases of arthritis of the rheumatoid type from whom articular tissues had been obtained for study were 7 having psoriasis. In 5 of the 7 cases the clinical and roentgenological findings and the pathological changes in the joints were indistinguishable from those of rheumatoid arthritis. The sixth case had, in addition to a widespread ankylosing arthritis, pronounced psoriatic involvement of the fingernails with marked arthritis of some of the terminal phalangeal joints. Although no tissue was obtained from these joints, the resected metatarsal phalangeal joints showed changes that were identical with those observed in rheumatoid arthritis.

The remaining case (a man 68 years of age) was of special interest. At 24 years of age he first noted arthritis in one terminal phalangeal joint. During the last 22 years of life he had complained of progressive arthritis of the terminal phalangeal joints of the hands and feet. He had been aware of the presence of psoriasis for 29 years. Psoriatic lesions of the nails were present. At autopsy it was possible to examine all of the joints of one hand and both feet and the majority of the other articulations. No lesions other than those of degenerative arthritis were found in any articulations except those of the fingers and toes. The majority of the terminal articulations were entirely destroyed and replaced by dense hyalinized scar tissue in which only minimal traces of inflammation could be detected. Extremely marked overgrowth of bone had occurred around the margins of the proximal ends of the terminal phalanges. The distal ends of the middle phalanges had undergone pronounced resorption. Bone atrophy, however, was not evident. The observed changes in these joints were unlike those of any usual form of chronic deforming arthritis. The dissimilarity between these joint lesions and those of rheumatoid arthritis was sufficiently great to suggest important differences in pathogenesis, if not in etiology. We have, however, been unable to eliminate the possibility that even these unfamiliar joint lesions may represent a rare form of rheumatoid arthritis.

Until additional information has been obtained, it is our belief that if the term "psoriatic arthritis" is to be used to designate a form of joint disease, its use should be restricted to cases such as this in which the arthritis is limited to the terminal digital joints.

HYPERTROPHIC PULMONARY OSTEO-ARTHROPATHY. A PATHOLOGIC STUDY OF SIX CASES. Edward A. Gall and Granville A. Bennett, Boston, Mass.

Abstract. Although clubbing of the fingers, one of the cardinal manifestations of hypertrophic pulmonary osteo-arthropathy, has been known since antiquity, it has only been comparatively recently that the wider distribution of the malady has been appreciated. It is our purpose to discuss the histopathology of the associated lesions, a phase of the subject which has received remarkably little attention. Material for this study was obtained by biopsy or necropsy from 6 patients who had, in addition to the osteo-arthropathy, in 4 instances pulmonary neoplasm, in 1 pulmonary emphysema, and in another, congenital heart disease. All had clubbed fingers and in 4 patients for whom roentgenograms of long bones were made, there was evidence of subperiosteal ossification to a varying degree in the tibia, fibula, femur, radius, ulna, metatarsals, metacarpals and proximal phalanges.

Histologic study showed that the periosteal lesion followed a well defined developmental pattern. Initially there was division of the periosteum into an outer fibrous zone in which an extensive lymphocytic infiltration was apparent and an inner edematous cambium layer. The swollen cells within the latter apparently made possible a deposit of osteoid upon the subjacent cortical bone. As the amount of osteoid increased, the deposits were arranged in a columnar fashion perpendicular to the bone surface. Calcification occurred in the deeper and older portion of the osteoid and fusion with the cortex took place. Osteogenesis was also noted to a marked degree in tendons at points of insertion. Continued ossification permitted the development of cancellous bony sheaths irregularly encasing the shafts of the involved bones. Intermittent periods of activity resulted in this sheath becoming laminated. Ultimately lacunar resorption of the original cortical compacta caused this also to become cancellous in structure and indistinguishable from the overlying subperiosteal bone. The thickness of the cortex thus became considerably greater than normal but it was exceedingly porous.

Specimens of clubbed digits from 4 cases exhibited minimal or no subperiosteal bone formation in the terminal phalanges. The clubbing evidently had resulted largely from edema and inflammatory infiltration of the soft tissues in this region. Joint tissues removed from all of the 6 cases showed in 4, slight to moderate edema and lymphocytic infiltration of the synovia and varying degrees of degenerative change in articular cartilage. None of these lesions could be considered specific.

It is concluded that hypertrophic pulmonary osteo-arthropathy in its fully developed form may be properly defined as that syndrome occurring as a sequela to a major visceral disease, usually intrathoracic in location, which is characterized by clubbing of the digits, ossifying periostitis mainly of long bones, and is frequently associated with joint manifestations. The sequence of events in the periosteal lesion has been described.

SPECIFIC THERAPEUTIC SHOCK—THE HUGH YOUNG REACTION. Ward J. MacNeal, New York, N.Y.

Abstract. When in the course of an established generalized bacterial infection, in particular with such organisms as staphylococci, colon bacilli or hemolytic streptococci, there is introduced into the blood stream an adequate

amount of suitable therapeutic agent, such as mercurochrome, antibacterial serum or bacteriophage, one may observe the production of a definite and often severe chill associated with and followed by a rise in temperature of 1° to 8° F., quickly succeeded by marked diaphoresis and defervescence and continued clinical improvement. This phenomenon, called by us (Sheplar, Adele E.; Spence, Martha Jane, and MacNeal, Ward J.: Serum therapy for infections with streptococci. *Arch. Surgery,* 1938, **37**, 772) the Hugh Young reaction, resembles somewhat the paroxysm of early tertian malaria. It is evidently associated with an active phagocytosis of the bacteria in the blood and with a reduction in number of the circulating neutrophilic leukocytes which seem to phagocytize the injured bacteria and to be themselves, in turn, phagocytized by endothelial cells in the spleen, liver, lymph nodes and bone marrow.

Discussion

(Dr. Eugene L. Opie, New York, N.Y.) Does this reaction resemble that which is induced by sanocrysin and other gold salts used in the treatment of tuberculosis? Sometimes following injection there was elevation of temperature of from 3° to 5°F. It was assumed for a time that the reaction could be controlled by antisera against products of tubercle bacilli, but this relation was not demonstrable.

(Dr. MacNeal) I do not quite understand the question, but I do not believe I could answer it, anyway. It was a question of the injection of something in the treatment of tuberculosis, with a rise in temperature.

(Dr. Opie) In some instances about 3 hours after injection of gold salts there was elevation of temperature.

(Dr. MacNeal) Undoubtedly it is a related reaction. I do not mean to say that this Hugh Young reaction has not many things in common with other reactions, but there is this peculiarity, that the substance injected into the body is known to have a deleterious effect, *in vitro,* on the microörganism present in the blood stream, or in a very active lesion in contact with circulating blood; and secondly, when we inject it in adequate quantity we get a sharp chill and a sharp rise in temperature, and a subsequent fall and improvement in the condition of the patient. Those are the criteria for this peculiar reaction. In the instance mentioned by Dr. Opie, the agent injected does not give this reaction for about 3 hours. The microbe is not present in the blood stream, either, and it seems to me, therefore, that it may be a related rather than an identical reaction.

ELECTRIC SHOCK: IMPORTANCE OF PATH, DISTRIBUTION AND DENSITY OF CURRENT IN DETERMINING SYMPTOMS AND PATHOLOGY. Leo Alexander and (by invitation) Arthur W. Weeks, Boston, Mass.

Abstract. A clinico-pathological and experimental study on electric shock was presented. Experimental study shows that electric current passes through the animal body as though it were passing through a structureless gel, always choosing the shortest path from contact to contact without deflection by anatomical landmarks. Living bone carries a similar amount of current, presumably because of the vascular bed which pervades living bone as completely as most other tissues of the animal body. A critical level for lasting disturbance with definite morphologic alteration of nerve tissue was found to be at 30 milliamperes per 3 mm. of nerve diameter for shocks of

5 seconds' duration. Lower values produce temporary disturbance, the duration of which is mathematically correlated to amperage and time. These experimental findings are applied to the interpretation of the clinico-pathological findings in accidental cases in man.

Discussion

(Dr. Howard T. Karsner, Cleveland, O.) This is a contribution of great significance. The description of the gel has included soft tissues. What information is there as to the passage of the current through bones?

(Dr. Alexander) We have found that bone passed the same amount of current as all other tissues, presumably because of the vascular bed which pervades living bone as completely as any other tissue.

(Dr. Alan R. Moritz, Boston, Mass.) I should be interested to hear a little more explanation of how the cows were killed.

(Mr. Weeks) The transformer on the pole broke down in very rainy weather; the current went down the wet pole and was not grounded in that area, because the underlying structure of the ground was sand and rock; therefore it flowed through the earth for a distance of three-quarters of a mile, and the cattle got enough current up their legs to cause their death.

(Dr. Alexander) The fact that the power company which owned the transformer paid damages for the death of those cattle is a point of confirmatory evidence. This was done after a very careful electro-technical investigation of the accident.

(Mr. Weeks) I might also point out that they were also all blooded cattle.

(Dr. Moritz) I suppose the wire fence might have had something to do with it.

(Mr. Weeks) Yes, the wire fence played a part in the picture. The fence was fastened to the pole which had held the faulty transformer, and the current went up through the fence. It is possible that one of the cows was killed by touching the fence, but the others were back of the fence and therefore they must have been killed by the current through the earth. It is not an unknown phenomenon. Horses have been killed by touching a third rail.

(Dr. Moritz) If an electric current tends to follow the pathway of least resistance, why did it deviate from its course to pass through the body of the cow?

(Mr. Weeks) The key to that situation is that the potential gradient is steepest near the point where it leaks out. If we apply that, we will have a high voltage near the pole, tapering off as it spreads away, and these cattle were near the pole. Therefore they were at the point nearest the greatest potential gradient.

(Dr. Jacob Werne, Jamaica, N. Y.) Were these cattle examined for lesions?

(Mr. Weeks) They were not.

THE LEUKOCYTIC RESPONSE IN EXPERIMENTAL SHOCK. Theodore J. Curphey and (by invitation) Eric Ponder, Mineola, N. Y.

Abstract. The polynuclear count (modified Arneth count) of Cooke is recognized to be a sensitive indicator of bone marrow activity, and it is known that a deflection of the count can be produced in rabbits under urethane anesthesia by crushing of muscle or bone, by irradiating with X-rays or

ultraviolet light in high intensities, or by the injection of extracts of macerated tissues. The stimulating agent in the extracts seems to be a protein. The deflection of the polynuclear count, conveniently expressed as a change in the figure for its mean, occurs within a few hours and passes off gradually, from 1 to 3 weeks being required for a return to the original steady state. Attempts have been made to assay the effect against that of a standard marrow stimulant such as nucleic acid injected under the skin of the back, but the deflection and return are too slow in the rabbit to allow this to be done.

In the guinea pig the polymorphs are much more complex, some of the cells having as many as ten nuclear lobes. This gives a right-handed polynuclear count with a mean of about 5.0 as compared with 2.0 to 2.5 in the rabbit. The marrow response to a standard injection (*e.g.*, 1 mg.) of nucleic acid is very short and transient, the maximum deflection occurring in about 2 hours and recovery being nearly complete after 5 hours. This makes it practicable to standardize the response of each experimental guinea pig to various amounts of nucleic acid, and such standardized animals may be used as test animals by receiving injections of the plasma of other guinea pigs which have been put into shock by the standard methods of bone-crushing, burning, etc. In the injured animals the polynuclear count becomes deflected to the left and the mean falls until it reaches about half its original value at the time when the animal shows circulatory collapse with unquestionable hemoconcentration. If about 1 cc. of the plasma of this animal is injected into a calibrated test guinea pig, the polynuclear mean of the latter falls, and the minimum, reached in about 2 hours, can be compared with the known response to nucleic acid and assayed in this manner. The test guinea pig, after receiving the plasma from the animal in shock, shows striking changes in behavior, accompanied by coldness of the feet and ears. As the polynuclear count is deflected, the polymorphs themselves show an altered cytology, the nucleus becoming foggy and ill-defined. This disappears as the count returns to its original state, which it does after a few hours, with minor irregularities sometimes showing themselves for 2 or 3 days.

The deflection of the polynuclear count is accompanied, generally speaking, by an increase in the total number of polymorphs per cmm., but the total count is so variable that we have discontinued using it in quantitative work.

Discussion

(Dr. Valy Menkin, Boston, Mass.) This paper is extremely interesting to me, because 2 years ago we were able to identify a leukocytosis-promoting factor in inflammatory exudates, particularly as encountered in animals that had a leukocytosis concomitant with inflammation. This factor has been shown to be a globulin, or at least to be associated with it. It has also been found in dog and rabbit exudates. Reifenstein of Syracuse has recently confirmed its finding in rabbit exudates. In unpublished work I have recovered it recently from human exudates. In other words, this factor can be identified in a number of different exudates produced by a variety of irritants in several animal forms. The evidence does not seem to point toward nucleic acid as the active factor encountered. We have in our studies been able to dispose of the nucleoproteins. In general the reaction elicited by the leukocytosis-promoting factor (LPF) is much more rapid than that obtained

with nucleotides. In the experiment of the above investigators, the femoral region is crushed. No doubt there is extreme local damage, and it therefore seems to me conceivable that the leukocytosis is referable to the liberation of the same type of factor as the leukocytosis-promoting factor. It would therefore be of interest to see whether one can isolate from such a damaged tissue this active globulin.

(Dr. Ponder) We are quite in agreement with you on the nature of the substance. In 1930 we pointed out that it is a protein; we did not go so far as to show it was a globulin. I am familiar with your work, and from my experience am able to corroborate such parts of it as I have tried. I hope I have not given the impression that because I used nucleic acid as the standard in the assays I think the substance produced in the animal is nucleic acid. I used to use thyroxin, but nucleic acid is more convenient, and that is the only reason I use it.

(Dr. Robert A. Moore, St. Louis, Mo.) May I ask Dr. Ponder if there was any difference in the response of individual animals of the same species? We have secured some evidence in mice that the response of the bone marrow to either Dr. Menkin's material or to nucleic acid is a part of the genetic constitution of that animal, and that some animals respond by a leukocytosis and some give no response at all.

(Dr. Ponder) As regards the mouse, we have not found it possible to use this method of assay, because a mouse's polymorphonuclears are peculiarly complicated, and very difficult to resolve into their parts. As regards the genetic constitution, I should not be at all surprised. Different guinea pigs give different responses and it is a source of some trouble; but our experiments are not extensive enough, in that our colony is not large enough, for us to be able to say whether there is any genetic effect. It would not matter, I imagine, in an individual assay.

CERTAIN PHYSIOLOGICAL DIFFERENCES BETWEEN SHOCK AND HEMORRHAGE. David R. Morgan, Marshall M. Lieber and (by invitation) Donald McGrew, Philadelphia, Pa.

Abstract. Comparison between animals in deep shock (intraperitoneal muscle implantation) and those dying from repeated hemorrhages shows that in the dogs with shock there develops a hemoconcentration of 36 to 40 per cent with an increase in the hemoglobin values, specific gravity, red blood cell count, white blood cell count, prolongation in the coagulation time and a marked fall in the sedimentation rate. There is a decrease in plasma volume, in plasma protein and in CO_2 content, an increase in the blood nonprotein nitrogen, and an inconstant blood sugar rise. Conversely, the blood of dogs after hemorrhage shows a distinct and immediate hemodilution with a decrease in red blood cell count, hemoglobin and specific gravity, a slowing of the coagulation time and an increase in the sedimentation rate. There is an increase in the plasma volume, the plasma protein, the plasma CO_2 and blood nonprotein nitrogen. The blood sugar shows an agonal rise. The lymph flow is increased in shock and decreased after hemorrhage. The parenchymatous tissues show a greater water content in shock than after hemorrhage. The urine in shock shows albumin, red blood cells, casts, bile salts and pigments and occasionally urinobilinogen. The urinary findings after hemorrhage are negative. There is a marked difference in the response

to hemorrhage at the critical blood pressure level of 80 mm. of mercury. Dogs with shock could be brought to the death point with a single small hemorrhage, whereas dogs following hemorrhage withstood subsequent bleeding until the production of exemia.

Discussion

(Dr. Max B. Lurie, Philadelphia, Pa.) May I ask how the blood volume was determined?

(Dr. McGrew) In this series we did not determine the blood volume In this current series, in trying to differentiate between shock and hemorrhage, no mention was made of the blood volume.

(Dr. Lurie) I mean the plasma volume.

(Dr. McGrew) We used the results of the work that has been done previously on normal dogs by Gibson at Harvard, in which he took normal dogs and correlated the plasma volume, the hematocrit reading and the hemoglobin. These figures, plus the direct hematocrit determination, made by taking the blood, citrating it and centrifuging it rapidly, which gives a rather gross determination of the plasma volume and also the amount of hemoglobin increase and the red cell content, give a fairly accurate indication as to the amount of plasma volume. The plasma protein we determined by the chemical analysis of the plasma.

(Dr. Stuart Mudd, Philadelphia, Pa.) I should like to point out that burns are apt to accompany conditions of shock and hemorrhage, and I want to express the hope that sometime before the morning is over we will have the pathology and physiology of burns integrated into the picture.

(Dr. Alexander S. Wiener, Brooklyn, N. Y.) I noticed that the water content of the tissue is given as high in one condition, and low in the other, but no figure is given for the normal.

(Dr. McGrew) We made no lengthy comparison with the normal. We did it in several dogs. We have not enough figures to give a series for normal dogs, such as we have for shocked dogs and for dogs following hemorrhage. The outstanding thing is that in practically every case the shock-tissue showed an increase in the amount of water over that contained in the hemorrhage-tissue. Whether the amount in the animal after hemorrhage is practically normal I cannot say at the moment. We can call it an increase over the hemorrhage value, or an increase over the normal.

(Dr. Theodore J. Curphey, Hempstead, N. Y.) In one of your experiments you had evidence to show that a small amount of hemorrhage in a shocked animal produced rather sharp change. Have you any evidence to show the effect of the shocking factor in an animal who is in the mid-stage of the experimental picture with hemorrhage — just the reverse of that phenomenon?

(Dr. McGrew) No, we have no experiment exactly like that, although we did combine the two to produce serious shock and serious hemorrhage in a series of dogs, and in these all I can tell you is that the blood pressure in every case fell rather dramatically and rapidly, but the physical findings and the chemical findings of the blood are those of the predominant factor; if there is more shock than hemorrhage you will get a shock-picture, although you can imagine with the combination of the two the blood pressure will fall rapidly.

(Dr. Virgil H. Moon, Philadelphia, Pa.) It has been one of the problems of shock that so many complicating factors are introduced. Hence in all of our work we try to avoid complicating factors and to test the effects of shock, so far as possible, uncomplicated by other agents. This work is a preliminary report on a comparison of shock and hemorrhage as separate items. There were only one or two points at which a complicating factor was introduced, and that was done intentionally. In dogs in shock we noted that only a small amount of hemorrhage was necessary to produce a fatal effect when the dog's circulatory deficiency had reached a critical level, whereas after hemorrhages it required an enormous loss of blood to bring the dog to a lethal point. I hope all present will realize we are trying to compare two separate items and to avoid complicating factors when possible.

STUDIES ON THE BLOOD HISTAMINE IN RABBITS DURING HEMORRHAGE, SHOCK PRODUCED BY MANIPULATION OF THE INTESTINES, AND FOLLOWING THE SUBCUTANEOUS INJECTION OF HISTAMINE. Bram Rose and J. S. L. Browne (by invitation), Montreal, Canada.

Abstract. The histamine content of the whole blood and of the plasma was studied in rabbits during hemorrhage, shock produced by manipulation of the intestines during ether anesthesia, and following the subcutaneous injection of histamine. The results obtained show that the general pattern of change, namely a decrease in the histamine content of the whole blood and an increase in that of the plasma, is the same in these three conditions. The degree of decrease in the histamine content of the whole blood is similar in all three, but there is a difference in time relationships. For example, in the case of intestinal trauma, the onset of shock is slower, and the decrease in the whole-blood histamine level is spread over a period of from 6 to 12 hours. In the case of hemorrhage, or the subcutaneous injection of histamine, the decrease is more rapid, coming on within an hour, and reaching a maximum within 2 hours. The increase in the histamine content of the plasma is greatest in those animals which were given a subcutaneous injection of histamine and less in that of the animals subjected to the effects of hemorrhage and intestinal manipulation.

From these findings, it thus appears that histamine may be in some way related to the shock syndrome in the rabbit, and to hemorrhage. It is as yet difficult to interpret whether the increase in the histamine content of the plasma or the decrease in the histamine content of the whole blood is the more significant. In this connection, however, it is interesting to note that anaphylactic shock is correlated with a rapid and marked decrease of the whole-blood histamine level in the rabbit (Rose and Weil, 1939), that the histamine content of the blood of patients in shock due to trauma or surgical interference is markedly decreased (Rose and Browne, 1940), and that the symptoms of histamine intoxication in patients following the subcutaneous injection of histamine may be correlated with decrease in the histamine content of the blood (Rose, 1940).

Discussion

(Dr. Virgil H. Moon, Philadelphia, Pa.) Dr. Dragstedt has demonstrated in anaphylactic shock that there is a marked increase in the histamine content of the blood, and in the cases of serious shock reported here there was

apparently the opposite — a fall in the histamine content. I should like to ask the authors whether either of them has comments to make on that situation.

(Dr. Rose) The experiments of Dragstedt were performed on dogs, and I think the work shows very well that the amount of histamine in the plasma of the dog increases considerably. In the rabbit, on the other hand, we have shown that there is a marked decrease of the histamine content of the blood during anaphylactic shock. I should like to emphasize, however, that the results presented today were obtained from patients with shock due to burns and in rabbits during hemorrhage and shock due to trauma.

(Dr. Moon) Then this feature as seen in the rabbit is opposite to that in the dog?

(Dr. Rose) Yes.

MORPHOLOGIC CHANGES IN EXPERIMENTAL SHOCK. Marshall M. Lieber and David R. Morgan, Philadelphia, Pa.

Abstract. Shock of varying degree and intensity was produced in dogs with diverse agents. These included tissue substances introduced intraperitoneally, burns, peptone poisoning, anaphylaxis, intestinal obstruction, roentgen irradiation of the abdomen, and others. The visceral changes after death in 113 dogs in this series included generalized capillo-venous congestion, most prominent in the liver, kidneys, gastro-intestinal mucosae, lungs and serosae. Frequently petechial hemorrhages were noted in these tissues and also in the epicardium, meninges and brain. When death was delayed, the soft tissues were edematous and there were effusions into the serous cavities. Histologically, capillo-venous congestion, edema and occasionally petechial hemorrhages were marked. Parenchymatous degeneration of the viscera was the rule. Focal necroses were noted in the liver, spleen, lymph nodes and adrenal glands in a number of animals. These morphologic features are directly related to capillary injury with increased permeability resulting in circulatory deficiency and anoxia of the tissues.

It is to be emphasized that the various tissues present a variety of changes which are dependent on several factors including the nature of the shock-producing agent, the intensity or quantity of that agent and the period of time over which it acts before death is produced. The severity of the pathologic changes does not always parallel the degree of shock produced. Under identical conditions of experimentation, the degree and distribution of the changes may vary in different animals of the same species.

Discussion

(Dr. E. T. Bell, Minneapolis, Minn.) I should like to ask whether the lesions produced are attributable to shock or the substances used to produce the shock. The focal necrosis in the liver and spleen, for example, would be a little more easily explained as the result of these toxic proteins that are liberated by the necrotic tissue.

(Dr. Lieber) We interpret these as probably due to the effect of these agents on the capillary endothelium, resulting in circulatory deficiency with anoxemia and anoxia of the tissues.

(Dr. Bell) Do these occur in any kind of shock?

(Dr. Lieber) We used all these various methods of producing shock, and

it did not matter which method produced it; the essential characteristics were the same, although they varied in degree.

(Dr. Alfred Plaut, New York, N. Y.) We saw calcification in one of the pictures presented to us; what about the time factor? How long after the shock took place were these animals killed, or did they die?

(Dr. Lieber) The 2 dogs that were burned lived until the eleventh day, at which time we killed them because of the discomfort they had from the burns on the body.

(Dr. Otto Saphir, Chicago, Ill.) I should like to know if you have any data on the postmortem findings on patients who have died as a result of shock, and if so, whether the changes are similar to those experimentally produced.

(Dr. Lieber) It is very difficult to interpret changes as they occur in human beings. It is not often we have the opportunity to examine the tissues sufficiently early after death, since postmortem changes occur so rapidly, much earlier in shock than in other conditions; so I would be very hesitant in interpreting changes as they occur in human patients unless I could obtain all the material within 2 hours after death.

(Dr. Tracy B. Mallory, Boston, Mass.) I should like to ask how many of the changes you have just described are present in animals in which a clinical degree of shock can be recognized and then are sacrificed some hours before they might otherwise die. If you sacrifice animals as soon as a clinical degree of shock can be recognized, do you find all these visible lesions?

(Dr. Lieber) No; the changes as they occur early are simply congestion and edema. With severer degrees of shock and early death petechial hemorrhages are found. The degenerative changes are seen in various viscera, but focal necroses as they occur in different tissues are usually associated with a severe degree of shock over a longer period of time; by that I mean over 10 hours.

THE TREATMENT OF SHOCK BY THE INTRAVENOUS ADMINISTRATION OF NON-HEMATOGENOUS MACROMOLECULAR SUBSTANCES. W. C. Hueper and (by invitation) G. J. Martin and M. R. Thompson, New York, N. Y.

Abstract. A rational treatment of shock must counteract not only the impaired circulation, the lowered blood pressure and the increased blood viscosity brought about by the escape of plasma through the vessel walls, but must obviate also the effects of the primary and secondary endogenous factors causing an excessive capillary permeability and a defective vascular tonus. The two macromolecular agents, plasma and gum arabic, used for this purpose not only fulfill relatively imperfectly these fundamental requirements, but offer also certain difficulties in their practical use because of limited availability, complicated preparation and handling, lack of standardization, acute allergic or toxic reactions, etc. Experiments were conducted with 0.5 to 1 per cent aqueous colloidal solutions of methyl cellulose fortified by the addition of the natural detoxicants, ascorbic acid, cystein hydrochloride, calcium glucuronate and glycine. These solutions were injected intravenously into dogs subjected to shock by the application of bags filled with crushed dry ice to the shaved skin or by the subcutaneous administration of histamine in an oil-lanolin emulsion. It was found that this medication

is efficacious and relatively safe, as it reduced quickly the hemoconcentration of the shocked animals in many instances; it prevented the development of the prognostically unfavorable marked leukopenia of cold shock; it seemed to have exerted a definitely life-saving effect in 3 dogs with histamine shock which exhibited leukocytoses between 55,000 and 63,000 cells, being in the lethal range, according to Moon. Whereas the osmotically active, macromolecular methyl cellulose solution cannot be considered as an adequate substitute for plasma, it possesses certain properties recommending it for further study as a more suitable non-hematogenous blood substitute than gum arabic. Methyl cellulose is produced in this country; it is easily sterilized by boiling and is refractory to bacterial infections of the ordinary type; much smaller amounts of methyl cellulose are necessary for producing a solution having the same viscosity as plasma than are required with gum arabic; methyl cellulose is more adaptable as it comes in several grades of viscosity reflecting differences in molecular size; it is not more hazardous as to secondary storage phenomena than gum arabic. The detoxicants added to the methyl cellulose solution apparently accentuated and supplemented the beneficial effect produced by the macromolecular agent.

Discussion

(Dr. Bram Rose, Montreal, Canada) I should like to ask if Dr. Hueper controlled any of these experiments by the injection of an equivalent amount of saline. You can prevent a certain amount of shock by the injection of normal physiological saline.

(Dr. Hueper) We have not injected normal saline because we do not think it is the solution which should be used in shock treatment. We need a colloidal macromolecular agent over a long period to sustain the plasma volume, which cannot be done by a medium like saline solution, or dextrose.

(Dr. Stuart Mudd, Philadelphia, Pa.) Have you made any attempt to apply this clinically?

(Dr. Hueper) No; our work has been only experimental.

(Dr. Greenblatt, New York, N. Y.) What is the specific rôle of ascorbic acid in these experiments?

(Dr. Hueper) It is well known that various detoxicants act rather specifically on certain toxic agents; ascorbic acid, for instance, detoxicates benzol and its derivatives; that is well known from a large number of experiments.

(Dr. Theodore J. Curphey, Hempstead, N. Y.) Have you had any toxic reactions in your animals?

(Dr. Hueper) No.

(Dr. Eric Ponder, Mineola, N. Y.) What is the osmotic pressure of the solution of methyl cellulose compared to that of plasma protein?

(Dr. Hueper) Practically isotonic.

SHOCK: PLASMA PROTEIN BUILDING IN EMERGENCIES AS INFLUENCED BY INTRAVENOUS DIGESTS. G. H. Whipple, Rochester, N. Y.

Abstract. By established technic for the measurement of plasma protein production in hypoproteinemic dogs, we have determined that an enzymatic (papain) digest of commercial casein given parenternally is as effective in plasma protein production as whole liver by mouth. This digest provides

materials needed to correct the hypoproteinemia as well as nitrogen for other body protein requirements. The dried digest is a golden yellow, granular material containing 12.5 per cent nitrogen. In a 5 per cent solution, sterilized by Seitz filtration, it is well tolerated when given either intravenously or subcutaneously.

Discussion

(Dr. Stuart Mudd, Philadelphia, Pa.) I think the utility of this procedure of feeding plasma proteins over a period of weeks is perfectly clear. I am wondering if Dr. Whipple has had any experience with it in such emergencies as burns, in which plasma may be given intravenously as an emergency measure for a week. Might it be possible to give plasma intravenously for a day or so, and to give such digests for the remainder of the emergency?

(Dr. Whipple) That is the hope we have, but a great deal of trial under clinical conditions will be necessary. Certainly this digest should be of value. It can do no harm, as we see it. How well it will be utilized, and how much plasma might be given under these abnormal conditions of shock I do not know.

(Dr. Alexander S. Wiener, Brooklyn, N. Y.) This work seems to open up many brilliant possibilities. One question I should like to ask is whether Dr. Whipple has any idea as to where the serum albumin is produced.

(Dr. Whipple) That is a nice question. My belief is that the albumin is produced in the liver, but others have different ideas. The evidence, I think, is accumulating that a good deal of the albumin, perhaps all of it, is produced in the liver. Certainly there are certain globulins (fibrinogen) produced in the liver, and the evidence indicates only in the liver. Other globulins appear to be produced outside the liver. Further than that one is on very debatable ground.

THE VASCULAR AND CELLULAR DYNAMICS OF SHOCK. Virgil H. Moon,* Philadelphia, Pa.

Abstract. Shock has been under intensive investigation for years, resulting in much information but not in complete agreement of interpretation. Confusion has resulted chiefly from four major causes.

1. Incomplete knowledge of the functions and reactions of capillaries delayed clarification of the problem. Endothelium is highly susceptible to the effects of various agents and conditions. These include bacterial substances, foreign proteins and split-products, extracts of normal tissues, histamine, bile, venoms, chemicals, poisons, metabolic products and even moderate lack of oxygen. Any type of injury to endothelium increases its permeability to plasma colloids. Leakage of plasma from the blood produces hemoconcentration, lowers the total blood volume and leads to a disparity between it and the volume capacity of the vascular bed. This disparity, if uncompensated, manifests itself in the characteristic signs of shock. The syndrome is accompanied by distinctive physiologic disturbances and pathologic features which are related directly to endothelial damage. Abnormal permeability of endothelium disturbs seriously the mechanism of water balance. Normal movement of fluid between the blood and the tissues depends upon several factors, including capillary blood pressure, osmotic pressure, concentration of electrolytes and others. But the action of these forces,

* By invitation of the Council.

in maintaining physiologic relations between intravascular and extravascular fluids, is absolutely conditioned upon the presence of a normal semi-permeable membrane—the endothelium—between them. Circulatory deficiency of this type is accompanied by vomiting, diarrhea, edema, hemoconcentration and by inability to absorb fluid from the tissues. These features indicate disturbances of fluid balance.

Abnormal *cellular* permeability also is an important feature of pathologic physiology. The outer surface of each living animal cell functions like a semi-permeable membrane. Hence each cell behaves as an osmotic unit. Living protoplasm maintains chemical concentrations differing markedly from those of the external fluids, but this property is reduced by any kind of cellular injury and is lost entirely as the cell dies. This allows the differences in concentration to be equalized and accounts for the high potassium content, hypochloremia and other chemical alterations of the blood during shock. This type of circulatory failure may develop after extensive traumatic injury, surgical procedures and burns. It occurs also incident to abdominal emergencies, intoxications, infections of unusual severity, serum disease and in acute poisoning of various kinds. When it follows trauma or surgical procedures, it usually is complicated by hemorrhage and by other factors, but it can be produced in uncomplicated form by various agents which injure endothelium. Adequate understanding of capillary reactions and function has removed one major obstacle to a comprehension of shock.

2. A second cause for confusion has been the failure to distinguish between shock and the effects of hemorrhage. Any type of circulatory deficiency activates the sympatho-adrenal system. This stimulates the myocardium, increases the strength and rapidity of the pulse, excites peripheral vasoconstriction, thereby causing peripheral ischemia, declining temperature and loss of tissue turgor. It causes an increase in the blood sugar, dilatation of the pupils and perspiration. Low basal metabolism, increased respiratory rate, thirst and declining blood pressure accompany both shock and hemorrhage. Hence several of the clinical signs are identical, but other accompanying features are opposite in character.

In shock the capillary endothelium becomes permeable, the tissue fluid and flow of lymph are increased, fluid balance is disturbed, ability to absorb fluid from the tissues is impaired, vomiting and diarrhea are frequent and infusions of fluids or transfusions of blood often are ineffective in treatment. None of these features result from simple hemorrhages.

In shock the blood becomes concentrated, the nonprotein nitrogen, potassium, calcium and magnesium contents are markedly increased, the sodium, chlorides and carbonates are decreased, the coagulation time is lengthened and the sedimentation rate is retarded. None of these changes are produced by hemorrhages.

During shock the urine is decreased in volume and contains albumin, erythrocytes, casts, bile and other abnormal substances. No characteristic urologic changes result from hemorrhages.

The necropsy findings after death from shock include edema, serous effusions, capillo-venous congestion, stasis and petechiae in the viscera, atony of the gastro-intestinal tract, focal necroses and acute degeneration of parenchymatous organs. None of these morphologic features result from the effects of hemorrhage.

It seems remarkable that these contrasts have escaped the attention of many who have discussed hemorrhage as related to shock. Hemorrhage, when present, is a highly important contributory factor, but distinctions between the accompanying physiologic disturbances invalidate the assumption that shock and the effects of hemorrhage are identical.

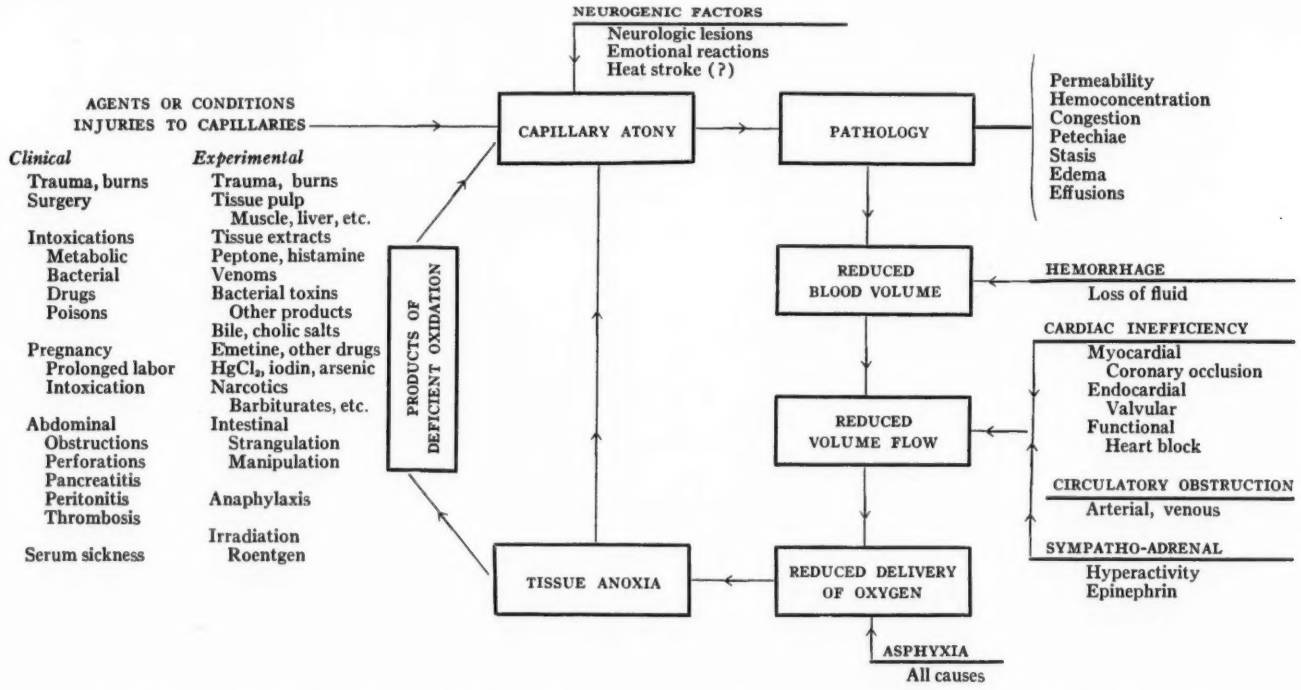
3. Factors of error inherent in experimental methods constitute a third major source for confusion. A method commonly used is to narcotize an animal deeply, then to produce extensive trauma to the tissues, using a decline in blood pressure as the indicator of shock. This method is open to serious objections. It is well known that the blood pressure may fall during shock or after hemorrhages or from deep narcosis. In some reports the workers showed conclusively that they were dealing almost entirely with hemorrhages; others recorded the marked depressor effects of the anesthetic. Under such conditions the result may be due to the narcotic, to absorption from traumatized tissues, to the associated hemorrhages or in part to each. This combination of indeterminate factors has led to undependable conclusions and to confusion.

Methods were devised for testing the effects of absorption uncomplicated by anesthesia and by hemorrhage. The implantation of tissue pulp into the peritoneal cavities of normal dogs produced regularly a circulatory deficiency having the characteristic features of the shock syndrome. This was accompanied by progressive hemoconcentration and by chemical alterations in the blood such as occur in traumatic or surgical shock and after extensive burns. Watery extracts of normal tissues produced similar effects when injected. These results confirm the interpretation that the absorption of products from damaged tissues is a factor in producing circulatory failure.

4. Finally, the belief that shock is purely a physiologic disturbance, unaccompanied by significant morphologic features, hindered the clarification of the problem. A characteristic pattern of morphologic visceral changes develops both in clinical and in experimental shock (*vide supra*). Such findings are indicative of endothelial damage and support the interpretation that various agents, including products absorbed from damaged tissues, exert deleterious effects upon capillary walls and thereby initiate a group of physiologic disorders leading to circulatory deficiency and to lack of oxygen in the tissues. This factor of itself causes endothelial permeability and thereby introduces a self-perpetuating feature which gives the mechanism the quality of a vicious circle shown diagrammatically on the following page.

Recent Theories

The Alarm Reaction. Selye described a syndrome resulting from severe damage but independent of the nature of the damaging agent. The term "alarm reaction" was applied to the sum total of the adaptive or defensive reactions against the effects of the damaging agent. The manifestations of shock were interpreted as due to inadequacy of the defensive reactions. When these are adequate, a second phase, "countershock," develops and is characterized by a reversal of the clinical signs of shock. This conception omits essential mechanisms from consideration. Shock, interpreted as inadequacy of physiologic defenses, presents an uncompleted picture. One still inquires by what mechanism is the circulation disturbed and why are the metabolism, renal function and chemical concentrations altered.



The relationship of various factors in the pathologic physiology of shock. The reciprocal effects of capillary atony and tissue anoxia give this mechanism the self-perpetuating quality of a vicious circle.

Sympatho-adrenal Hyperactivity. The excessive effects of epinephrine will produce the complete syndrome of shock. This may result from tissue anoxia produced by maximal arterial constriction. Freeman has proposed that shock following trauma, surgery or burns similarly may result from hyperactivity of the sympatho-adrenal system evoked by pain, emotions or injury to the tissues. The theory is questionable in several particulars. It has not been shown that the animal's own glands can produce enough epinephrine to cause shock within the time limits of the experiment. Neither excessive pain nor prolonged stimulation of nerve trunks will cause shock, nor will cutting the cord or severing all nerve paths from the traumatized area prevent its development. Others have reported that animals subjected to adrenalectomy or to sympathectomy are as susceptible to shock from trauma or other agents as are normal animals. Activity of the sympatho-adrenal system is maximal during physical combat, yet this does not cause shock independent of injuries.

Roentgen irradiation of the abdomen causes delayed necrosis of intestinal mucosa accompanied by delayed shock. This treatment is painless and provides no cause for delayed sympatho-adrenal hyperactivity.

Evidence supporting this theory was derived from a comparison of the effects of hemorrhages in sympathectomized and in normal dogs. Since shock and the effects of hemorrhage are not identical (*vide supra*) these experiments do not bear directly upon the problem. If the author could show that sympathectomized animals are resistant to the effects of several shock-producing agents, he would increase greatly the weight of his evidence.

THE RÔLE OF POTASSIUM IN THE SURVIVAL TIME AFTER BILATERAL NEPHRECTOMY. S. Durlacher and D. Darrow (by invitation) and M. C. Winternitz, New Haven, Conn.

Abstract. The survival time after bilateral nephrectomy varies considerably, but in general far exceeds that after renal artery ligation. This has been shown to be related to the rise in the potassium of the blood. When animals are fed on a low potassium diet until the potassium content of their tissues and serum has been reduced, their survival time following bilateral nephrectomy is significantly increased over control animals fed a normal diet. In the latter the nonprotein nitrogen of the blood rises rapidly and the serum potassium is found to be at fatal levels at the time of death, whereas in the former the animals die after a prolonged period, with markedly elevated nonprotein nitrogen and serum potassium levels that are not in the fatal range.

DISUSE ATROPHY OF RENAL ARTERIES. E. T. Bell, Minneapolis, Minn.

Abstract. In chronic glomerulonephritis atrophy of the renal cortex may result from extensive obliteration of the glomeruli, and in chronic pyelonephritis destruction of the tubules in the medulla or corticomedullary junction may bring about atrophy of areas of the cortex. In neither of these diseases is the cortical atrophy due to vascular obstruction, but the arteries supplying the cortical scars may show appearances which are easily confused with primary arterial disease.

This alteration in the arteries is due to the circumstance that their function is reduced to a minimum, and the change may appropriately be called

"disuse atrophy." The lumen of the affected vessel is reduced in size and its walls appear relatively thick in proportion to the size of its lumen. The media has a glassy, semihyaline appearance. The medial change is due to medial fibrosis — a replacement of smooth muscle by collagenous fibers. In the afferent arterioles this is the only alteration, but in the small arteries one sees, in addition to medial fibrosis, a marked folding of the internal elastic lamina corresponding to the decreased size of the lumen. In the medium-sized arteries there may be an elastic intimal thickening, which is independent of hypertension since it occurs in chronic pyelonephritis without hypertension. Uncomplicated disuse atrophy of small arteries and arterioles differs from primary vascular disease in the absence of intimal changes.

Discussion

(Dr. M. C. Winternitz, New Haven, Conn.) As Dr. Bell has pointed out, it is very desirable indeed to distinguish between uterine and renal artery changes. The fibrosis of the media of small arteries and arterioles, emphasized by Dr. Bell, is important. I do not think the evidence is at all conclusive that it is a manifestation of disuse atrophy. By narrowing the ureters, medial necrosis followed by fibrosis may be produced. Different viewpoints exist concerning the fate of the nephron when the glomerulus or a part of the tubule is destroyed. Tubules may persist, as has been shown by Dr. Oliver and his associates, even in infarcts. This is important evidence.

(Dr. Bell) The only thing I can say is that in this form of disuse this is the type of change we see. Whether it can be produced in some other way or not I do not know. Probably it might result from some other cause. As to the nephron not disappearing when a part of it is destroyed, I am aware of Dr. Oliver's work, but I have never been able to find, in tracing serial sections, any such thing as that — if a glomerulus is hyaline you invariably find it associated with an atrophic tubule.

UREMIA AND PERICARDITIS. FINDINGS AS THEY RELATE TO HYPERTENSION. Leon K. Baldauf and (by invitation) Robert E. Ingersoll, Cambridge, Mass.

Abstract. The primary and fundamental function of the kidney is the regulation of the inorganic composition of the plasma rather than the obvious excretion of nitrogenous waste products. In uremia with pericarditis there is always marked nitrogenous retention in the blood, a high blood pressure, a persistent acidosis and a tendency to hemorrhage. Blue and co-workers maintained that the azotemia was due to a loss of salt. Causes for this deficiency include kidney failure and cardiac failure with accumulation of fluid in the cavities, use of diuretics, particularly acid salts and mercurials, vomiting, and insufficient chlorides in the diet. This marked nonproteid nitrogen retention and finally dehydration with further concentration of salts in the serous cavities account for the granular and crystalline deposit on the pericardial surface which we consider the primary and essential pericardial lesion.

The adrenal cortex is frequently spoken of as supplying the water-salt hormone. In Addison's disease and in adrenalectomized animals the amount of nonproteid nitrogen is greatly increased. Indeed, in many cases, the rise of nonproteid nitrogen and the corresponding fall after restoration with

cortical extract have been employed in assaying the strengths of different lots of extract. The normal sodium content of the blood plasma in adrenal insufficiency is usually decreased about 15 per cent and chloride content somewhat less, both phenomena being due to excretion of these substances in the urine. There is a simultaneous increase of potassium and magnesium. There is a close similarity of the blood picture of adrenal insufficiency and of chronic nephritis. With the loss of sodium chloride, there is in both conditions an increase in the potassium and magnesium serum content. Equally important are changes noted in the kidney tubules in adrenalectomized animals. These changes noted by many observers were first described by Marshall. The relation between activity of the adrenal and blood pressure is significant. The low blood pressure in Addison's disease and adrenalectomized animals, the increased blood pressure in hyperactivities and following the injection of synthetic preparations, and the reduction in blood pressure in the Goldblatt hypertensive cases following adrenalectomy have been frequently noted. Smith, calculating from plasma clearance (diodrast) and the filtration of water from the blood in the glomeruli (clearance of inulin), demonstrated in early renal hypertension an early loss of tubular function. The increased glomerular pressure is dependent on constriction of the efferent arterioles, which increases the filtration pressure and the flow of blood through the kidney. Following this vasoconstriction and ischemia, afferent arteriolar changes occur. That the vascular changes in the afferent vessels are secondary to hypertension and not primary appears likely for many reasons.

While the conservation of salt and water is due essentially to its reabsorption in the kidney, this is not the only organ where such reabsorption occurs. There is reabsorption of cerebrospinal fluid by the blood sinuses, of intraocular fluid, of salt and water in the gallbladder and of salt and water in the large bowel. In all these organs responsible for reabsorption we have hypertensive vascular lesions. The pancreas is intimately related to salt metabolism and with adrenalectomies performed years ago a constant pancreatic lesion was noted. We believe that the activities of the adrenal and pituitary are responsible for the reabsorptive processes everywhere. While comparisons are admittedly difficult and hazardous, the similar changes in vessels, for instance, in the glomerulus and choroid plexus and the marked disturbances in a main function common to both organs (the reabsorption of water and salt) make the suggestion of a similar etiology of hypertension in these organs seem natural.

As expressed by F. P. Parker, the preservation of the normal chloride is essential to the maintenance of the proper physical equilibrium of the various electrolytes of the tissues and body fluids which influence the distribution of water between the circulatory blood and the tissues. The reabsorption of water and salt to permit the excretion of nitrogenous material is the combined function of normal kidney tubules and the adrenal gland. Failure of either tubules or adrenal cortex will result in loss of salt, accumulation of nitrogenous products and a reconstitution of the various electrolytes in the blood. Addison's disease follows destructive lesions or atrophy of the adrenal gland and a failing hypertensive kidney is characterized by decompensation and hyperplasia of the kidney tubules. Failure of the kidney either in essential or secondary hypertension is dependent on tubular decompensation. With tubular failure, and the loss of sodium

chloride, hyperactivity of the adrenal follows in the attempt to establish salt equilibrium. Hyperactivity of the adrenal cortex as a compensatory effort to overcome the loss of salt from decompensated tubules may thus give rise to secondary hypertension, while the hyperactivity of the gland itself with failure of the reabsorptive processes may lead to what is generally known as essential hypertension.

Discussion

(Dr. M. C. Winternitz, New Haven, Conn.) In Dr. Ingersoll's comprehensive review of medicine, I did not understand what type of pericarditis he had in mind — whether it was fibrinous or another variety.

(Dr. Baldauf) Many of the cases of pericarditis are not fibrinous in nature. Covering the pericardial surfaces is a fine granular and crystalline material. We believe this to be the essential and primary lesion. The fibrinous lesion we feel is secondary to this lesion: in some instances due to a terminal infection; in other instances to a chemical irritation with high blood urea and a tendency to hemorrhage.

RENAL BIOPSIES FROM HYPERTENSIVE PATIENTS. Benjamin Castleman and (by invitation) Reginald H. Smithwick and Robert S. Palmer, Boston, Mass.

Abstract. During the past 4 months we have had the unique opportunity of studying renal biopsies removed from 16 hypertensive patients who were being subjected to sympathectomies. The selection of patients for sympathectomy is very difficult and one of the purposes of this study was to learn how much renal damage, if any, there actually was in patients with relatively early hypertension who had no clinically demonstrable renal impairment, and conversely, how extensive the renal damage was in patients with renal insufficiency. During the course of the operation, usually following the nerve resections, a wedge-shaped biopsy approximately 6 to 7 mm. wide and about 5 mm. deep was taken. When biopsies were taken from both kidneys, the gross and histologic appearances on each side were essentially similar.

The patients included in this series were young individuals, only 2 being in their forties and the others ranging from 20 to 38 years of age. Their blood pressures were over 200 systolic and the symptoms of hypertension had been present on the average for 2 years, although in 3 patients the history went back for only a few months and in 3 others for 10 to 12 years. All showed some retinal change. Eleven out of the 16 had normal renal function as measured by the PSP test. Except for 1 patient who should not have been selected for operation, none of them approached the stage in their disease where demise seemed imminent.

The kidneys were fully exposed, found to be normal in size and only rarely were the capsules unduly adherent to the cortex. Occasional tiny scars were visible in about half the cases but most of the parenchyma was smooth and looked normal to both surgeon and pathologist. The biopsies were usually taken through normal-appearing cortex and in a few cases included a portion of a scar.

When one examines microscopically the kidney removed at autopsy from the average hypertensive patient, including those not dying from renal

failure, there is no doubt, even at low magnifications, of the presence of vascular disease. The striking feature in this series of biopsies was that at first glance many of them appeared normal, but when searched for, vascular disease was evident in every biopsy. The vascular lesions found were classified as intimal hyalinization, medial hypertrophy and degeneration and endothelial hyperplasia. No necrotizing arteriolitis was observed—further confirmation of the theory that this lesion is a terminal change. Most of the cases showed combinations of these types, but in a few one type was predominant. No specific type of disease was limited to any one size of vessel, although many of the diseased small arterioles showed only medial hypertrophy. There were 4 cases, 2 with a definite history of infection, in which the biopsies showed focal scarring consistent with pyelonephritis according to the criteria of Parker and Weiss. The vascular disease, however, was not limited to the scars, although it was much more severe in those areas. Independent efforts to grade the severity of the process by clinician and pathologist showed a fair degree of correlation, although in general there was a tendency for the clinical grades to be more severe. Valid criticism of these gradings might be made on the basis that the biopsy was too small to be representative of the whole kidney. We admit this possibility, but since most of the kidneys appeared uniform at operation and were the same on both sides, we feel that each biopsy was a fairly good sample.

From these findings it is quite obvious that there is evident renal vascular disease in hypertensive patients long before there is any clinical suggestion of renal failure or any serious morbidity. These findings, therefore, do lend some support to the theory that the renal disease precedes the hypertension. On the other hand, there are perhaps some clinical results that are pertinent to this problem. Before this small group of patients was studied, 30 hypertensive patients essentially similar in all respects to the present group, were subjected to the same kind of sympathectomy. This operation consists of a bilateral total splanchnic denervation, the great splanchnic nerves being removed from the semilunar ganglion to approximately the midthoracic level and the sympathetic trunk resected from D-9 to L-1 or L-2 inclusive. These 30 patients have been followed for periods of 3 months to 2½ years with the following results: Sixty per cent of them now have normal or near normal blood pressures, most of them responding immediately following the second stage of the operation. Although no renal biopsies were taken in this series, it is reasonable to assume that had they been made, renal pathology similar to that found in the recent biopsied series would have been observed. It is much too early to make any statement on the follow-up of the biopsied cases, but it is also reasonable to infer from the experience of the previous larger series that, since 14 out of the 16 biopsied patients had an immediate response postoperatively, most of them will be markedly benefited by the operation in spite of their definite renal disease. This is merely a preliminary report indicating anatomic findings only and no attempt is made at this time to fit it into any theory as to the cause of hypertension, or even to suggest that the vascular disease may be reversible. Further data such as tests for renal blood flow preoperatively and postoperatively, pressor substance determinations from the peripheral and renal veins which are now being collected from these patients, and the condition of their kidneys in years to come may shed further light on the subject.

Discussion

(Dr. M. C. Winternitz, New Haven, Conn.) I think this is an important and interesting contribution. It has been difficult to understand just why sympathectomy should be effective clinically. The demonstration of the changes in the renal artery is interesting. Dr. Castleman has made a conservative and clear presentation of the facts.

(Dr. Joseph E. Smadel, New York, N. Y.) Would Dr. Castleman care to discuss early changes observed in the glomerular tufts of his sections?

(Dr. Castleman) These biopsies were done during the last few months and we expect to go further in the examination of these kidneys for early changes. In most of the cases, however, the glomeruli appeared perfectly normal. No diagnostic early changes were noted.

(Dr. Irving Graef, New York, N. Y.) Pertinent to this report is a case I had the opportunity of studying last year and upon which I reported at the Pittsburgh meeting. It has since had an interesting follow-up. In the study which I was making, evaluating Goormaghtigh's claims of the so-called juxtaglomerular apparatus, a kidney was obtained by surgery from a female 30 years of age who was pregnant and who had had hematuria for a period of 5 years. Because her obstetricians were loathe to carry her through pregnancy with the hematuria, fearing some obscure complication, exploration was advised. The urologist exposed a grossly normal kidney and on splitting the pelvis revealed the presence of blood, but no obvious changes as the source of it. Small blocks and the rest of the kidney were fixed *in toto* at once. Histological sections studied serially revealed the presence of massive medial hypertrophy affecting the entire arteriolar tree and extending backward for a considerable distance into the interlobular arteries. The patient at the time had no hypertension. She had been hospitalized on five previous occasions and on none of them had shown any hypertension. She had no impairment of renal function, except the hematuria from the left kidney. We could not account for the vascular change. It corresponded with the changes described by Dr. Goormaghtigh in some of his cases of hypertension, but this patient did not have hypertension.

In May, after I had reported this case at the Pittsburgh meeting, the patient suddenly developed eclampsia, with hypertension, and was delivered of a living child. Subsequently her hypertension disappeared and today she is normal, a little more than a year after the nephrectomy. I cite this case again at this point simply to indicate that the renal arteries may behave in a remarkable manner, exhibiting marked medial hypertrophy, without hypertension. Whether this patient will develop hypertension I do not know, but I feel we have a great deal yet to learn about the behavior of medial muscle under various physiological and abnormal conditions. This contribution of Dr. Castleman's is consistent with everything we know about established hypertension in which we are certain that a number of mechanisms are operating to affect the vasculature of the body, not only the media, but the intima as well. As Dr. Winternitz and his associates have shown, we can dissociate many of the effects of this curious renal disease, some of them leading to necrosis of the vessel walls, some to changes in the capillaries, and some to changes in the smooth muscle. I believe it would be wrong for us to keep on simplifying our concept of the relationship of hypertension to arteriolar disease and ascribe every instance of hypertension to similar changes in the vessels, or all changes in the vessels to hypertension.

THE MORPHOLOGICAL ASPECT OF THE GOORMAGHTIGH CELLS (JUXTAGLOMERULAR APPARATUS) IN THE NORMAL AND DISEASED HUMAN KIDNEY. William Kaufmann (by invitation), Albany, N. Y.

Abstract. Following a previous preliminary report, approximately 400 kidneys removed surgically and at autopsy were examined for the presence of the juxtaglomerular apparatus or "Goormaghtigh cells," as we shall call the cells in recognition of the man who first described them. Various fixing and staining technics were used and Goormaghtigh cells were found regularly at the vascular pole of many but not all glomeruli, along the vas afferens and even along the vas efferens and the interlobular artery in kidneys of patients ranging in age from 3 months to 75 years.

Special attention was given to cases of progressive arteriolar nephrosclerosis, malignant nephrosclerosis and chronic pyelonephritis with secondary vascular changes, all with clinical evidence of hypertension. In these cases hypertrophy and hyperplasia of Goormaghtigh cells were uniformly observed, but the finding of increased acidophilic or basophilic cytoplasmic granules, as seen in experimental renal ischemia in dogs by Goormaghtigh, and in rabbits by Dunihue and Candon, could not be substantiated. The preservation of Goormaghtigh cells in cases of advanced sclerosis of the arterioles appears to support Goormaghtigh's experiments, in which feeding of massive doses of vitamin D_2 or calciferol to dogs produced arteriolar necrosis in the renal arterioles, but left the cells of the juxtaglomerular apparatus intact for a long time. In the absence of definite cytoplasmic granules, the hypertrophy and hyperplasia of Goormaghtigh cells in our patients with hypertension constitute the only morphological basis for their possible relationship to the formation of a renal pressor substance.

Discussion

(Dr. Francis Bayless, Cleveland, O.) I should like to congratulate Dr. Kaufmann on his very beautiful kodachromes. In Cleveland we have had opportunity to examine the kidneys of a number of Dr. Goldblatt's animals —monkey, dog and goat—and some material from hypertensive human beings. In the animal series there were normal kidneys, kidneys that were ischemic, and kidneys contralateral to an ischemic kidney. The animal material we studied was received as unknown; the tissue was fixed in Bouin's, in Zenker-formol, or in formalin, and stained by various methods. We can confirm the observations of Dr. Goormaghtigh, Dr. Kaufmann, and others, and the chief point of interest now is the interpretation of the findings. In some instances the juxtaglomerular apparatus appears to be larger in renal ischemia and in some instances larger in hypertension. The presence of this apparatus in the normal kidney is quite easy to establish by anyone who cares to look. The most troublesome thing is the inconstant finding of granules in the cytoplasm of the *Polkissen* cells. They vary in number, depending on the species of the animal. In human beings they seem to be very hard to find, although they are encountered. In some animals they are more common, and seem to have a relationship to the hypertensive state or to renal ischemia. The further question to be decided concerns the possible relationship between enlargement of the juxtaglomerular apparatus and the presence or absence of so-called Ludwig's vessels in the kidney. We are now trying to establish whether that vessel is normally present in the kidney,

and if so, if it becomes altered in disease. Our method combines the use of fixation and staining of one kidney with arterial injection of the opposite kidney with neoprene (a colloidal synthetic rubber mass), and then comparison to correlate gross and microscopic details.

(Dr. Irving Graef, New York, N. Y.) It is very pleasant indeed to see Dr. Kaufmann's material. He began some of his studies on this apparatus in this laboratory, and my own initial interest in the juxtaglomerular apparatus began from seeing some of his random preparations made in animals. My own experience in human material has given me great misgivings concerning the validity of observations based on some postmortem material, because fixation makes a great difference in the appearance of medial cells. Even an hour's lapse of time in a body which has been obtained at a temperature of 98.6° F. may make a difference in the appearance of the medial cells, and I have seen postmortem artefacts of all sorts which have troubled me greatly in trying to determine whether the cell arrangements were the results of fixation, or postmortem change, or both. In the few normal human cases obtained promptly and fixed adequately I have seen cells quite like those Dr. Kaufmann has demonstrated, but their random distribution and the tendency to find these cells near the outermost glomeruli with greater frequency than in the inner ones has puzzled me very much. I have concentrated rather on dogs; and I think Dr. Bayless and I have been looking at the same material, because Dr. Goldblatt was kind enough to send me biopsies of unknowns from his dog material too. I can substantiate all of Dr. Bayless's remarks with reference to the dog, and make one addition. The granular cells which are not visible in man, except perhaps once in a hundred cases, in the dog increase in ischemia. Where we are dealing with 2 kidneys, 1 normal and 1 ischemic, if the normal is exposed to hypertension we may find no more than the normal distribution of these granular cells, whereas in the contralateral ischemic kidney they are easy to find, and are obviously increased in number. Again they appear in the preglomerular portion with greater frequency, but only in the cortical glomeruli. Why this is so I do not know. It was observed by Zimmermann in 1933 in many animals, and has been confirmed by Goormaghtigh, that the outer glomeruli appear to possess an architecture which is different from the deeper glomeruli. Embryologically they are different and that may have something to do with it. But it also suggests that renal function varies with individual nephrons. It may be that these changes are purely the result of ischemia in the terminal glomeruli.

So far as the clear cells are concerned, I wonder whether Dr. Kaufmann and Dr. Bayless have any idea about the relationship of that clarity to tonus. Is it not possible that the clear cells are relaxed cells which are fixed in a state of relaxation, while the cells showing myofibrils and granular structures may be contracted cells fixed in that state?

(Dr. Kaufmann) There is nothing that I can add to Dr. Bayless's remarks. As Dr. Graef pointed out and I mentioned in my paper, it is extremely difficult to see any granules in the Goormaghtigh cells of the human kidney. Only very recently, and that means in the past few days, using different fixing fluids and different staining methods, we think we have been able for the first time to see cytoplasmic granules more clearly than before. That is all I can say on this subject at the present time.

However, in the experimental animal, such as the mouse and the cat,

it is very easy to discover granules in the Goormaghtigh cells and, as Dr. Bayless mentioned, anybody who cares to look for them will find them. The difference between the myofibrils of the smooth muscle cells of the arteriolar media and the granules of the Goormaghtigh cells is readily visible in most instances. Also the granules are extremely large in comparison to the fine mitochondria of the tubular epithelial cells. They have a more rounded appearance and look somewhat like the granules of an eosinophilic leukocyte.

From the studies of our human material it is not possible at the present time to draw any definite conclusion as to the possible function of these cells. Two possibilities have to be considered at present: one, that the Goormaghtigh cells are functional units, responsible for the opening and closing of the preglomerular arterioles by a swelling and relaxation mechanism, possibly thus regulating the glomerular bloodflow; or second, that they are endocrine cells, as Goormaghtigh pointed out in his recent paper, which might be responsible for the production of a renal pressor substance. Investigations in this direction are being carried out presently to clarify these two theories.

"Anti-Renin" and Other Evidences of Tolerance to the Various Substances Contained in Extracts of Kidney. E. Mylon and R. Katzenstein (by invitation) and M. C. Winternitz, New Haven, Conn.

Abstract. Animals injected repeatedly and over a long period with extracts of kidney always show the same vasopressor reaction (renin effect). This may be masked by a more prompt vasodilatation or a protein shock reaction, as can be proven by removal of the vasodilator substance from the extract or suppression of the shock reaction by desensitization. Some evidence for increased tolerance against the necrotizing effects that follow ischemia of the kidney is demonstrable.

Functional Structures in Renal Tumors. Walter Schiller, Chicago, Ill.

Abstract. Most investigators have attempted to solve the much debated problem of the origin of the so-called Grawitz tumor by identifying the cellular elements of this tumor as either renal or adrenal in morphological character. Attempts to prove either the renal or adrenal origin of this tumor have both been somewhat successful on the basis of this criterion, and consequently no final decision could be reached. However, the presence of functional structures in these tumors similar to those found in the kidney during physiological activity or under pathological conditions is of far greater importance than mere morphological identity or similarity. Structures of this type are demonstrated and discussed in this paper. The storage of protein has been observed in the renal tubules, either as hyaline droplets in nephrosis, or as blood pigment in hemoglobinuria. The same change can be found in benign and in malignant renal tumors (hypernephromas). This tends to prove the renal character of this tumor tissue. On the other hand, however, these findings may help to determine whether in pathological kidneys the storage of protein is due to secretion, absorption or degeneration. Since the histological arrangement in these tumors is such as to rule out secretion or degeneration as the possible etiology of renal protein storage, absorption must be looked upon as a probable etiology of this pro-

tein storage. When the epithelial lining of the benign as well as of the malignant tumors piles up and fills the lumen, the cells lose their polarity and become transformed into adrenal-cortex-like cells. The histological and cytological pictures reveal that with the loss of polarity, another latent prospective potency, the potency to differentiate into adrenal cortical elements, is awakened. These tumors consequently must be traced not to misplaced, definitely differentiated renal or adrenal cells, but to latent, prospective, neoplastic renal, or adrenal potentialities hidden in tubular cells which cannot be distinguished from normal renal cells by routine microscopy. Under the stimulus of chronic inflammation or sclerosis, the latent potentialities are aroused; the renal character is manifest first and gives rise to papillomatous formations, but later, after proliferation and solidification have eliminated cellular polarity, the adrenal cortex potency becomes manifest.

HISTOGENESIS OF EXPERIMENTAL ADENOCARCINOMA OF THE SMALL INTESTINE IN MICE. Harold L. Stewart and (by invitation) Egon Lorenz, Bethesda, Md.

Abstract. Adenocarcinoma of the small intestine was induced in mice by oral administration of aqueous olive oil emulsions of either 1, 2, 5, 6-dibenzanthracene or 20-methylcholanthrene. The growths occurred at all levels in the small intestine, the majority lying from 15 to 20 cm. from the pylorus. The tumors were composed of atypical glands derived from the intestinal mucosa. All coats of the intestine were permeated by the neoplastic tissue. Metastases occurred to the pancreas, base of the mesentery and mesenteric lymph nodes. Excised fragments from several tumors were successfully transplanted subcutaneously into mice of the same strain as the animal in which the tumors originated and have been carried for many generations without change in histologic structure. To date a large number of mice have been autopsied in which the intestinal changes occurring during the stages of development of the carcinoma could be followed in detail.

Polypoid carcinoma of the small intestine occurred rarely under these experimental conditions in contrast to the frequency with which this form of neoplasm is observed in the large intestine in man. Of intermediate frequency but still rare was the development of carcinoma at the site of a lymph follicle in the small intestine. In this location the mucosal glands grew down into the follicle and into the underlying muscle, resulting in fissures and complete destruction and disappearance of the lymphoid structure. The latter was replaced by an area of atypical glands which completely permeated the intestinal wall.

The most frequent changes observed in the intestine during the evolution of the neoplastic process were as follows: At first there was hyperplasia and downgrowth of the basal glands of the mucosa into the lamina propria. A number of glands appeared to be affected simultaneously over an area approximately 1 mm. in width. The downward growth of these glands into the submucosa did not progress by way of lymphatic vessels. Instead the lymphatic vessels in the submucosa became constricted and ultimately obliterated due to the proliferation of reticulum and collagen in the submucosa about them. The cells lining the infiltrating basal glands became more atypical and were sometimes several layers thick. Individual cells

contained droplets of mucin and tended to lose their polarity with relation to one another, presenting at this early stage the atypical features characteristic of the cells of a fully developed carcinoma. Occurring independently of, or in conjunction with, these changes there was the development of atypical hyperplastic changes more superficially in the mucosa. The earliest departure from normal noted in this situation were solitary small areas composed of a few irregular acini. These were lined by hyperchromatic cells which were smaller than the adjacent normal cells, the nucleus almost completely filling the cell. The stroma between the glands became more compact resulting in shrinkage and depression of the surface. The innermost layer of the deep musculature immediately beneath these hyperplastic glandular areas became thickened due to proliferation of smooth muscle cells. These newly proliferated muscle cells grew upward to meet the infiltrating glands which were progressing downward. This led to disappearance of the submucosa and muscularis mucosae in the area of involvement and permitted direct contact between the glands and the smooth muscle cells. After this contact was established the two muscular coats and the peritoneum became permeated with the infiltrating mucosal glands, resulting in a picture easily recognizable as carcinoma.

NEUROFIBROMATOUS TUMORS OF THE EARS OF RATS PRODUCED BY PROLONGED FEEDING OF CRUDE ERGOT. Arthur A. Nelson and (by invitation) O. Garth Fitzhugh and H. J. Morris, Washington, D. C.

Abstract. About 100 albino rats were fed crude ergot in dosages of 5, 2 and 1 per cent of their diet for 15 months to 2 years, with the intention of testing possible chronic toxic effects. After about 1 year, tumors, histologically neurofibromas, began appearing on the ears. Most of the animals on 5 per cent ergot, a few on 2 per cent and none on 1 per cent developed neurofibromas; they occurred only on the ears. Of several hundred similar rats of equal age treated with a variety of food and drug dyes and solvents, only 1 has developed a similar tumor, also on an ear. Tumors other than neurofibromas have occurred in both the ergot-fed and other groups with their usual spontaneous frequency. No gangrene has been caused by the ergot feeding; the alkaloid content of our dosages is probably much too small to produce gangrene. Pathological changes in the viscera have been minor. The fraction of the ergot responsible for tumor production has not yet been determined.

Discussion

(Dr. Otto Saphir, Chicago, Ill.) I would like to ask whether any attempts have been made to transplant the tumor.

(Dr. Nelson) No.

(Dr. Arthur W. Wright, Albany, N. Y.) I would like to know whether or not Dr. Nelson has carried out these experiments in more than the one strain of rats.

(Dr. Nelson) No. Our animals were the ordinary albino Osborne-Mendel strain of rats.

(Dr. Wright) Did any tumors arise spontaneously in animals that had not received ergot?

(Dr. Nelson) Our rats have about the usual number of spontaneous tumors for their variety. Most of you will recall a paper by Curtis and

Bullock reporting several hundred spontaneous rat tumors in a colony numbering thousands; our incidence is about the same as theirs, and our ergot animals have had just their share of the tumors and no more.

(Dr. Wright) Were any of these spontaneous tumors fibrous tissue tumors?

(Dr. Nelson) Of three dozen, about 6 were, and they were more of a malignant spindle cell sarcoma type, with none of the features of neurofibroma; that, of course, opens up the big question as to whether these spindle cell sarcomas originate from perineural fibrous tissue. One rat had a much more benign fibroma. Only 1 rat out of hundreds had a neurofibroma similar to those reported here and the tumor was also on an ear.

(Dr. Antonio Rottino, New York, N. Y.) What was the dosage of ergot?

(Dr. Nelson) The doses given were 1, 2, and 5 per cent of the diet; the ergot was ground in small quantities so that it would not lose its potency and mixed with the diet. It was learned that the animals would do better if the 5 per cent doses were worked up to over a period of about a month.

(Dr. James H. Peers, Washington, D. C.) I would like to ask if there was any necrosis of the ears before the tumors appeared.

(Dr. Nelson) No; in the very earliest stages the ear appeared normal, and then a very small elevation developed, but there was no necrosis or anything of that nature until after the tumors became larger.

HISTOLOGICAL CHANGES PRODUCED IN ORAL SQUAMOUS CELL EPITHELIOMAS BY FRACTIONATED EXTERNAL IRRADIATION. John W. Hall and (by invitation) Milton Friedman, New York, N. Y.

Abstract. A histological study of 28 cases of squamous cell epitheliomas treated by fractionated irradiation is presented. Each case had from 3 to 9 biopsies, including 1 before treatment. Special emphasis was placed on irradiation keratogenesis.

THE NATURE OF SO-CALLED ANGIOBLASTIC MENINGIOMAS — SCLEROSING HEMANGIOMAS OF THE MENINGES. Orville T. Bailey, Boston, Mass.

Abstract. Angioblastic meningiomas have been regarded as tumors of blood vessel origin. However, their fat and pigment content and particularly their neuroglial element have served to set them apart from other hemangiomas. In view of the consequences of sclerosis in cutaneous hemangiomas it appears that the angioblastic meningiomas are hemangiomas in which a similar process of sclerosis has occurred. The sequences of pigment and fat deposition are identical with those in the cutaneous form. The meningeal tumors extend for a distance into the underlying brain. The neuroglia in the neoplasms results from participation of the ectodermal supporting tissue in the process of sclerosis. This is confirmed by finding identical tissue sequences in hemangiomas which are located wholly within the brain substance.

THE NATURE OF "SOLITARY OR EOSINOPHILIC GRANULOMA" OF BONE. Sidney Farber, Boston, Mass.

Abstract. This report deals with results of studies, made in part with the collaboration of my clinical colleagues, Dr. William Green and Dr. Leo J. McDermott, concerning the nature of certain benign, destructive, solitary

or multiple lesions in the bones of 10 children whose progress has been followed for from 3 to 10 years since the recognition of the first lesion. These appeared to be identical with what has been described in the recent literature as "solitary granuloma" by Otani and Ehrlich, and "eosinophilic granuloma" by Lichtenstein and Jaffe, and by Hatcher. They involved mainly the flat bones, particularly the skull, ribs and pelvis, although the long bones did not escape. Roentgenological examination revealed round, oval or irregular destructive lesions often with a punched-out appearance which suggested either myeloma, tumor metastases or Schüller-Christian's disease. The presenting sign was usually either swelling or pain. Healing occurred readily under X-ray therapy or after curettage, and to some extent spontaneously. Pathologic examination revealed a granulomatous process in which eosinophilic infiltration was frequently, but not always, a prominent feature. Foci of necrosis without evidence of bacterial inflammation or suppuration were commonly present. Stimulation of the bone marrow was so marked in some instances that the diagnosis of myelocytic myeloma was excluded only with difficulty. Large mononuclear phagocytes, sometimes in giant cell formation, dominated the picture. These contained débris, remnants of destroyed bone, and often finely divided lipid which was stainable by Scharlach R, and was only rarely doubly refractile. Bacteriologic examination and a limited number of animal inoculation studies revealed no evidence of an infectious agent. No attempt has been made to isolate and identify a possible filtrable virus. Eosinophilia (6 per cent) was demonstrated only once. Blood cholesterol values were essentially normal. The total blood fat was significantly elevated in one half of the patients. Nine patients are alive and apparently in good health with no evidence of visceral disease; no details could be obtained concerning 1 patient who died outside the hospital. Comparison of the pathologic material obtained from these 10 patients with lesions in the skeleton and viscera in Schüller-Christian's disease and with several examples of what is known in the recent literature as "Letterer-Siwe's" disease, and study of recorded descriptions of the evolution of bone lesions in Schüller-Christian's disease have led us to the conclusion that all three conditions represent variations in degree, stage of involvement and localization of the same basic disease process. There is no implication in the foregoing statement that any one of these three conditions is a xanthomatous process or a manifestation of a primary alteration of lipid metabolism. These studies do not lend support to the conclusion that "eosinophilic or solitary granuloma of bone" is either a new or a separate disease entity. If this suggestion concerning the nature of these benign bone lesions is correct, caution must be exercised in prognosis because of the possibility of later visceral involvement.

Discussion

(Dr. Louis Lichtenstein, New York, N. Y.) The material which Dr. Farber has presented is very interesting indeed. However, it is difficult from his presentation to accept his interpretation of the condition in question as an expression of Schüller-Christian's disease. In fact, he did not define his criteria for the anatomic diagnosis of Schüller-Christian's disease.

Apparently, Dr. Farber holds that the finding of a few foam cells in an occasional eosinophilic granulomatous lesion establishes a link between the

latter condition and Schüller-Christian's disease. We have had the opportunity of examining biopsy material from 12 cases of eosinophilic granuloma. (Incidentally, all but 1 of these showed only a single bone lesion.) None of the cases which we have seen showed collections of foam cells as a prominent feature of the lesion, and altogether the cytology of the lesion is not the cytology regarded as characteristic for Schüller-Christian's disease. One observes areas of focal necrosis in the lesions of eosinophilic granuloma. Furthermore, in most of the lesions, there is very appreciable and often heavy infiltration of eosinophilic leukocytes, which may be so concentrated as to simulate micro-abscess formation. Moreover, the macrophages which constitute another conspicuous feature of the lesion, as Dr. Farber has emphasized, contain phagocytosed red blood cells and eosinophiles, iron pigment and eosinophilic granules. That is, they are not essentially lipophilic macrophages, and while some may take up a very slight amount of lipoid granules, many contain none.

Clinically, too, eosinophilic granuloma is different from Schüller-Christian's disease. The former sets in abruptly, sometimes with a mild febrile reaction, and a good percentage of the patients also present an eosinophilia ranging between 4 and 10 per cent. The bone lesions develop rapidly and large destructive lesions may already be present within a few weeks. In cases with multiple lesions, most of these lesions seem to appear all at once, like a shower, which they seem not usually to do in Schüller-Christian's disease.

(Dr. Henry L. Jaffe, New York, N. Y.) I want to ask Dr. Farber whether any of the cases he described showed involvement of the dura, pleura or periosteum — features which are supposed to be prominent in the pathologic anatomy of Schüller-Christian's disease. It seems worth while to cite here, as a piece of evidence against Dr. Farber's conception of eosinophilic granuloma of bone as a form of Schüller-Christian's disease, the case of a girl 8 years of age now under observation at our hospital. Six weeks prior to admission she complained of moderate generalized abdominal pain and when she was permitted to be out of bed (a week later) it was noted that she had a mild limp on the right side. X-ray examination of the right hip region 1 week later showed an area of rarefaction in the neck of the right femur about ¾ of an inch in diameter. This lesion increased rapidly in size, and additional lesions have been found in the following sites: shaft of left femur, ninth right rib, and second and ninth left ribs. While in the hospital she has shown a leukocytosis and a mild febrile reaction. Two lesions have been explored, and the curetted tissue revealed a picture identical with that which Dr. Lichtenstein and I had previously described under the heading of "eosinophilic granuloma of bone." The lesions did not show collections of foam cells, fibrous tissue reaction to the lesion, walling-off reaction of the bone around the lesions, or any other feature which pathologists in the past have come to recognize as belonging to the pathologic anatomy of Schüller-Christian's disease. I cannot understand how a condition can be held to represent Schüller-Christian's disease (even as a variant) when it has a totally different pathologic anatomy and clinical course.

(Dr. Paul Gross, Pittsburgh, Pa.) I should like to say that from a study of this condition, of which we have one example, and from a study of the literature, Dr. Harold Jacox and I have come to a conclusion identical with that given by Dr. Farber. The foam cells which in many cases of Schüller-Christian's disease have been stressed as important diagnostically, have been

absent in a considerable number of cases of Schüller-Christian's disease. Consequently the regrettable statement has appeared repeatedly in the literature that a biopsy in Schüller-Christian's disease is not necessary because such characteristic cells may not be found. The eosinophilia which is considered to be practically pathognomonic of eosinophilic granuloma is also frequently a very prominent feature of Schüller-Christian's disease, and the eosinophilia of the peripheral blood in cases of eosinophilic granuloma may be duplicated in many cases of Schüller-Christian's disease. The lesions in both diseases may be associated with considerable fibrosis.

I think one lesion of a xanthomatous character with which Dr. Farber is familiar, but probably did not have time to mention, is the so-called solitary xanthoma of bone, of which numerous examples exist in the literature and which can very readily be placed in the same category as so-called eosinophilic granuloma and Schüller-Christian's disease.

(Dr. Sadao Otani, New York, N. Y.) May I ask if there was any history of trauma among your cases? Most of our cases have had a definite history of trauma. One of the cases we observed was a boy who was hit on the skull by a baseball. The granulomatous lesion developed at the exact site of trauma. A biopsy of this lesion showed the typical histology. None of our cases showed lipoid cells which to me are histologically characteristic of Schüller-Christian's disease.

We are fully familiar with the picture in Schüller-Christian's disease. We observed one particular case from the beginning to the end of the disease, *i.e.*, from the initial lymph node biopsy to autopsy. It was true that the biopsied lymph nodes showed practically no lipoid cells; nevertheless we suggested the diagnosis of Schüller-Christian's disease because of other histologic criteria. Gradually, however, the patient began to show typical bony lesions on X-ray examination. When the patient died 9 months after the first biopsy, at autopsy we found fully characteristic lipoid granulomatous lesions of Schüller-Christian's disease.

We did not overlook a similarity between the solitary granuloma and Schüller-Christian's disease. In fact, when in 1932 one of our cases of granuloma was first observed, Dr. Klemperer seriously considered that the lesion might belong to those seen in Schüller-Christian's disease. None of our cases, however, showed lipoid cells and they pursued clinical courses different from the cases of Schüller-Christian's disease. Therefore, the idea that the granulomatous lesions might belong to the lipoid granulomatosis group was given up by us, and we finally came to the conclusion that we were dealing with a discrete granulomatous lesion whose cause is thus far unknown. One of the cases we reported did not reveal conspicuous numbers of eosinophilic leukocytes in the lesion, although it was otherwise typical of this granuloma. Therefore, we prefer the term "solitary granuloma" to "eosinophilic granuloma."

(Dr. Farber) To answer Dr. Otani first concerning the relation of fracture to the lesion, I may say that trauma of importance was found in the history of several cases. In one instance a boy was struck on the head by a ball, and X-ray examination showed a punched-out defect in the skull which had been there obviously for some time antedating the injury.

I am very much interested in the observations of Dr. Gross. I certainly add solitary xanthoma to this group of lesions, although I did not mention it. We have had no evidence of visceral lesions in the group of 10 patients I

described today. In 1 patient who has had twenty-five bone lesions in the last 10 years, a yellowish nodule appeared on the leg several months ago. This, on histologic examination, showed much the same picture that was described today.

For many years we experienced difficulty in interpreting the lesion. It was not until transitional stages were encountered that the nature of the process became clear. I believe one difficulty is that we have too narrow a conception of Schüller-Christian's disease. This might well be expected since the disease picture was described originally on roentgenological and medical grounds and not on the basis of pathological examination. I might add in passing that I am not willing to accept the classification of Schüller-Christian's disease in the group of lipid metabolic diseases. In regard to the problem at hand we have been forced to the conclusion, on the basis of pathologic studies and information gained from the clinical and roentgenological characteristics of the disease process, that eosinophilic granuloma and lipo-granuloma are but variations of the same process, and that eosinophilic or solitary granuloma of bone, Schüller-Christian's disease and Letterer-Siwe's disease differ from one another only in the degree and site of involvement and the duration of the process. Further studies will be necessary before the etiologic factor can be defined.

LOBULAR CARCINOMA IN SITU — ONE OF THE RARE FORMS OF MAMMARY CANCER.* Frank W. Foote (by invitation), New York, N. Y.

Abstract. Mammary carcinoma *in situ* has been recognized for many years, but the term, *in situ,* has not been used. This long-recognized form has been designated "noninfiltrating comedo-carcinoma." The type of mammary carcinoma to be presented here differs from this type in that it takes origin not from the larger duct system but from the component parts of the mammary lobule.

The lesion in the pure, noninfiltrating form is quite infrequent, only 2 such examples being encountered in a consecutive series of 300 primary operable mammary cancers. There is no way in which a clinical diagnosis of lobular carcinoma *in situ* can be made. Even in excised breast tissue, gross features of mammary cancer are missing and one must depend upon microscopic examination for diagnosis. The microscopic changes include an abrupt alteration in lobular cytology featured by the appearance of "pagetoid" cells. The lesion occurs in multiple lobules that may be several centimeters apart, and hence when this pattern is found it is hazardous to stop treatment short of simple mastectomy. One case is cited in which local excision was done. The significance of the histology was not appreciated and within a few months this patient had infiltrating mammary cancer with metastases to axillary nodes and skeleton. How long lobular mammary carcinoma may remain *in situ* cannot be definitely stated, but its duration for 1 year without infiltration has been seen in 1 case. As soon as infiltration occurs, the gross appearance of lobular carcinoma becomes similar to that of many infiltrating mammary carcinomas. Microscopically, however, the pattern of infiltration is characteristic and easily recognized after some practice. The discovery of this type of growth pattern in a good many mammary cancers promotes the belief that infiltrating lobular mammary carcinoma is more frequent

* This article appears in full in this issue. See p. 491.

than might be suspected from the apparent rarity of the *in situ* lobular form of the disease.

EARLY CANCER OF THE GASTRO-INTESTINAL TRACT. William Carpenter MacCarty, Sr., Rochester, Minn.

Abstract. From 1917 to 1940 inclusive, I have routinely measured all resected malignant and benign tumors. I believe it is fair to associate earliness and lateness with size and the presence or absence of involvement of lymph nodes. This review deals only with cancers of the stomach and large intestine because they are among the most frequent sites of cancer. I have arbitrarily taken 2.5 cm. in diameter as the criterion of smallness or earliness. Table I is a summary of data originally determined for each of the years, but condensed here so as to include only the totals for the 24 years.

TABLE I
Small Cancers (2.5 cm. in Diameter or Under)

With lymphnodal involvement			Without lymphnodal involvement		
Stomach	Rectum, sigmoid, rectosigmoid	Rest of colon	Stomach	Rectum, sigmoid, rectosigmoid	Rest of colon
52 of 1162	27 of 1140	6 of 322	141 of 834	106 of 1683	11 of 433

TABLE II
Summary of Measurements of 6474 Resected Cancers

	Stomach	Rectosigmoid, rectum, sigmoid	Rest of colon
Number	2408	3102	964
Average size	6 cm.	5.7 cm.	7 cm.
Percentage with lymphnodal involvement	62	37	38
Largest	19 cm.	20 cm.	15 cm.
Smallest	0.5 cm.	0.9 cm.	1 cm.
Percentage of all 2.5 cm. in diameter or under	9.7	4.7	2.2

Summary. The statistics suggest that there has been some improvement in recognition of early cancerous lesions by the general medical profession. The greatest improvement is seen in the stomach, the rectum and rectosigmoid, all of which regions are now becoming generally accessible to more frequent direct vision and X-ray study. The large intestine from the ileum to the rectosigmoid is still a region for roentgenoscopic improvement, although the figures show that there has been a probable improvement.

READ BY TITLE

UNILATERAL RENAL ATROPHY AND HYPERTENSION. A. B. Baggenstoss and (by invitation) Nelson W. Barker, Rochester, Minn.

Abstract. This study includes all cases (84) of unilateral renal atrophy which have come to necropsy at the Mayo Clinic. There were 48 cases of pyelonephritic atrophy, 28 cases of hydronephrotic atrophy and 8 cases of pyone-

phrotic atrophy. Fourteen cases of unilateral hypoplasia also were studied. Death in most of these cases was due to neoplastic disease or infections of various types. Hypertension was a cause of death in only 5 cases. The incidence of hypertension was determined for the different types of renal atrophy and also for a control group of 100 consecutive cases of similar age distribution in which necropsy was performed. The incidence of hypertension in this latter group was 29 per cent. Only in the cases of pyelonephritic atrophy (39.6 per cent) and pyonephrotic atrophy (37.5 per cent) was the incidence of hypertension greater than in the control group. The incidence of hypertension was 41.9 per cent for the cases of pyelonephritic atrophy in which the atrophied kidney weighed 75 gm. or less and 35.4 per cent for the cases in which the atrophied kidney weighed between 75 and 110 gm.

In 26 of the 56 cases of pyelonephritic or pyonephrotic atrophy there was some degree of active inflammation in the atrophic kidney. In the scars of the kidneys in which there was pyelonephritic or pyonephrotic atrophy, there was generally arteriosclerosis grade 2 or 3 (on the basis of 1 to 4, in which 1 designates the mildest and 4 the most severe condition) while in the non-scarred portions, when these were present, the arteries were as a rule normal or revealed less sclerosis than those in the scars. This was true of the cases in which there was hypertension as well as those in which there was not hypertension.

In 14 of the 56 cases in which there was pyelonephritic or pyonephrotic atrophy, there was some degree of active inflammation in the opposite kidney. Inflammation in the opposite kidney was more often present in the cases in which there was hypertension than in those in which there was not hypertension. The degree of arteriosclerosis in the opposite kidney was generally less severe than in the atrophied kidney and in 10 of the 22 cases in which pyelonephritic or pyonephrotic atrophy was associated with hypertension the arteries and arterioles in the opposite kidney were considered normal for the age of the patient.

Although the results are of questionable statistical significance because of the small number of cases, they suggest that unilateral pyelonephritic or pyonephrotic atrophy is associated with hypertension more often than one would expect on the basis of chance. They also suggest that hypertension is more likely to be present if the degree of atrophy is severe.

BLASTOMYCOSIS: SPREAD AND TISSUE REACTION IN THE HUMAN. Roger D. Baker, Durham, N. C.

Abstract. Twenty-two cases of blastomycosis from which histopathological material was available, including 4 complete autopsies, were analyzed with regard to the primary infection, spread of the infection in the body, and tissue reaction. The tissue reaction is contrasted with that of tuberculosis and analyzed in relation to iodide therapy, reactions to skin and complement fixation tests, and extent and duration of the disease.

CHRONIC PORTAL OCCLUSION WITH ANEURYSM OF SPLENIC ARTERY AND CARCINOMA OF LIVER (HEPATOMA). Milton D. Bosse and James M. Strang (by invitation), Pittsburgh, Pa.

Abstract. Chronic portal occlusion associated with aneurysm of the splenic artery and carcinoma of the liver (hepatoma) in a white male, 58 years old,

is reported. The occlusion of the portal system was by calcific thrombi and probably resulted from acute appendicitis. The aneurysm of the splenic artery was of the cirsoid type, the vessel measuring 1.5 to 2 cm. in diameter and 55.5 cm. in length when it was straightened. The hepatoma was associated with portal cirrhosis.

THE PRESENT INCIDENCE OF TUBERCULOUS INFECTION. William H. Carnes (by invitation), New York, N. Y.

Abstract. The prevailing concepts of the pathogenesis of adult tuberculosis are based on the fact, established in the early part of this century, that virtually every city-dwelling adult has at some time acquired a tuberculous infection. That this is still a fact in the United States today has been questioned on the strength of the results of tuberculin surveys on large bodies of students in various communities in the past 10 years. These suggest that perhaps as much as half the population now reaches adult life without having acquired an infection. However, with the recently accumulated evidence that the tuberculin test may fall far short of establishing the true incidence of infection, it becomes desirable to gain more certain information from a postmortem anatomical search. Such a survey has been made on cases autopsied at the Baltimore City Hospitals and the Johns Hopkins Hospital in the years 1938 to 1940. The series includes only patients dying of diseases other than tuberculosis in which the entire lungs, together with all the bronchial and tracheal lymph nodes, were available for detailed search with the aid of the X-ray. All lesions were identified grossly and all were examined microscopically unless perfectly typical. The criteria for the diagnosis of their tuberculous origin were the same as those used in similar reported investigations in the past. The results of the routine autopsy examination, including that of the mesentery, were also utilized. A total of 536 cases was examined. Of these 307 were white and 229 negro. There was a fairly equitable distribution of the cases over the various age groups from 2 months to 89 years.

The results show that, as in the past, there is a rapid rise in the proportion of infected individuals from infancy to adult life. The greatest increment in the percentage of infected individuals occurs in the period from 5 to 15 years of age. During this period approximately 40 per cent of the population sampled acquired a tuberculous infection. The results also indicate that probably less than 30 per cent of Baltimore's population reaches the age of 20 years without having had an infection. All of 114 individuals over 60 years of age showed evidence of infection. No significant differences were revealed between the white and negro groups in any of these respects.

A comparison of these data with the results of a similar investigation by Opie in St. Louis in 1916 has been attempted. Serious limitations are imposed by the paucity of cases in several age groups of that study. However, when those results are examined by the same standards as the contemporary data, it appears probable that in the population examined by Opie not more than 15 per cent reached the age of 20 years without having acquired an infection. The approximate nature of all these estimates must be emphasized. Convincing proof that the incidence of tuberculous infection in St. Louis in 1916 was different from that in Baltimore in 1940 is lacking. No support is offered for the thesis that the incidence of tuberculous infection in city-dwelling adults has changed drastically in the past 20 years.

EFFECTS OF THE CONTINUED ADMINISTRATION OF SULFATHIAZOLE AND SULFAPYRIDINE TO MONKEYS. David R. Climenko (by invitation) and Arthur W. Wright, Albany, N. Y.

Abstract. Sulfathiazole and sulfapyridine, in doses from 0.5 gm. per Kg. per day up to 10 gm. per Kg. per day, were administered to monkeys for a maximal period of 28 days in order to obtain some evidence of the comparative toxicity of these two compounds. Drugs were administered by stomach tube as milk suspensions at 8-hour intervals throughout the entire period of medication. Daily observations of blood concentrations were made.

At a dose level of 0.5 gm. per Kg. per day, animals receiving sulfapyridine died on the 13th, 14th and 24th days of medication, respectively. Hematuria was present in all. At autopsy, urolithiasis, degenerative changes of the tubular epithelium, particularly of the collecting tubules, pyelitis and cystitis were observed. Monkeys receiving the same dose of sulfathiazole showed no ill effects during the 28 days of medication. One animal of this latter series, sacrificed on the 29th day for necropsy, showed no significant pathological changes other than slight edema of the kidney and a chronic inflammatory process of the renal pelvis.

The difference between sulfathiazole and sulfapyridine disappeared when the dose level was increased; at and above 1.0 gm. per Kg. per day fatalities occurred and severe renal lesions were observed in both series. These manifested themselves as parenchymatous and fatty degenerative changes of the epithelium of the convoluted and collecting tubules associated with the presence of crystalline material in the latter; focal necrosis and focal inflammation, and ulceration, necrosis, and desquamation of the epithelial elements of the larger collecting tubules. The renal pelves showed acute inflammatory reactions associated with submucosal hemorrhages. The severity of the lesions varied directly with the height and duration of the concentration of the drug in the blood. It should be pointed out that the dose range employed in this series approximates 10 to 200 times the usual therapeutic range.

BRAIN CHANGES IN PERTUSSIS. Vera B. Dolgopol, New York, N. Y.

Abstract. Neurological complications occur in pertussis in small children and are fatal in a large proportion of cases. The most frequent histologic finding is the "eosinophilic" (ischemic) degeneration of hippocampal pyramids and of Purkinje cells. Multiple scattered hemorrhages and lymphocytic plugs in capillaries and veins are next in frequency. No perivascular cuffing is observed. Loss of myelin is seen very rarely, apparently as a result of compression and secondary degeneration of myelin within areas of perivascular hemorrhages. The process is not an encephalitis, but rather an encephalopathy. No *Haemophilus pertussis* or virus was found in several brains examined. The pathogenetic basis for the brain lesions in pertussis, according to several authors, is stasis in the cerebral circulation.

THE PRODUCTION OF ANTIBODIES IN THE POPLITEAL LYMPH NODE OF THE RABBIT. W. E. Ehrich and (by invitation) T. N. Harris, Philadelphia, Pa.

Abstract. Various antigens were injected subcutaneously beneath the plantar surface of the foot, and after various intervals lymph was collected from the single efferent lymph vessel of the popliteal lymph node. Agglutinin,

hemolysin and complement-fixation titers were determined in lymph of regional and opposite lymph nodes and blood serum. These titers were compared with the number of lymphocytes in the lymph and with the weight and histologic appearance of the popliteal lymph nodes. The results of these experiments support the concept that antibodies are formed in lymph nodes and that lymphocytes play a considerable rôle in antibody formation.

CYSTIC FIBROSIS OF PANCREAS. I. H. Erb, Toronto, Canada.

Abstract. This communication is concerned with cystic and fibrous changes in the pancreas, usually of young infants, associated with some type of pulmonary infection, in many cases of the nature of bronchiectasis. In some instances there is also metaplasia of bronchial epithelium.

EXPERIMENTAL STAPHYLOCOCCIC PNEUMONIA IN RABBITS. Istvan A. Gaspar, Rochester, N. Y.

Abstract. In order to study experimental lung lesions produced by staphylococci, a series of rabbits was injected intratracheally with virulent *Staphylococcus aureus*. A 5 cc. saline suspension of a 24-hour growth of hemolytic *Staph. aureus* (2500 million organisms per cc.) was injected into each rabbit. One rabbit was killed every day up to the 12th day, then 1 on the 16th day and on the 20th day. The rabbits developed tracheitis, bronchitis and bronchogenic lung infection very similar to that observed in human lungs. The infection did not kill the rabbits. The initial hemorrhagic consolidations were followed by small and large gray consolidations and by small abscesses seen microscopically. The consolidations involved one third to one half of each lung. Staphylococci were recovered from the lungs in pure culture up to 8 days after injection. After that day the lung cultures became completely negative. Masses of staphylococci were seen in the lungs microscopically during the first days after the injection. They disappeared gradually. The small abscesses became absorbed later and by the 20th day only congestion, some fibrosis and the final stages of repair were present. These experiments showed that although the rabbit lung appears to possess better resistance to *Staph. aureus* than the human lung, nevertheless they demonstrate that *Staph. aureus* can cause bronchogenic pneumonia and lung abscesses without the association of any other organisms or virus. It is hoped that the action of some of the new drugs on *Staph. aureus* pneumonia can be tried with similar experiments.

Sterile saline and *Staph. aureus* vaccine were also injected intratracheally in other rabbits to determine the lung pathology produced by these agents.

GLOMERULONEPHRITIS OF RATS FOLLOWING THE ADMINISTRATION OF SULFAPYRIDINE. Paul Gross and (by invitation) Frank B. Cooper and William A. Morningstar, Pittsburgh, Pa.

Abstract. Relatively large doses of sulfapyridine were administered by stomach tube daily for 17 to 67 days to white rats from which one and one-half kidneys had been removed. Similar medication was also given to a group of unilaterally nephrectomized rats and to a group of normal, unoperated rats. The gross and microscopic pictures of urolithiasis medicamentosa with hydronephrosis or hydro-ureters were encountered in only 20 per cent of all animals in the three series.

A nonexudative glomerulonephritis was found in some rats. This was most frequent and severe in the animals from which one and one-half kidneys had been removed, while the unoperated animals exhibited the least involvement and relatively slight or early lesions. The unilaterally nephrectomized animals occupied a position intermediate in amount and severity of renal involvement. The glomerular lesions consisted of thickening of basement membranes of glomerular capillaries, hyaline degeneration, focal necrosis, endothelial proliferation of glomerular tufts and focal adhesions of tufts to Bowman's capsule in foci of capsular epithelial proliferation.

Sulfapyridine did not seem to be the direct cause of these lesions. It appears more likely that the production of the lesions was related to the excessive work required of the glomeruli which remained after surgical resection of renal tissue and the consequent obstruction by sulfapyridine uroliths. The glomerular lesions observed resemble those caused by nephrotoxic sera or by high protein diet.

ENVIRONMENTAL FACTORS INFLUENCING FEVER IN PULMONARY TUBERCULOSIS: A STATISTICAL STUDY. John S. Howe and (by invitation) Alvin Mayne, Richmond, Va., and Chicago, Ill.

Abstract. The percentile occurrence of fever in the population of a tuberculosis sanitarium was determined daily for a period of 14 months. Seasonal, weekly, and daily trends were identified and correlated statistically with various environmental factors, such as visiting days, the environmental temperature, the barometric pressure, etc. The results and their significance are discussed.

EXPERIMENTAL COLLOID DROPLETS IN RENAL EPITHELIUM. Frederick Johnson (by invitation) and Hans Smetana, New York, N. Y.

Abstract. The kidneys of urodeles have two types of nephrons: (1) "closed" nephrons which are similar to those in mammals; (2) "open" nephrons which communicate with the peritoneal cavity by means of the "nephrostomial canal" which is lined by ciliated epithelium and opens into the proximal portion of the convoluted tubules. Materials injected into the peritoneal cavity reach the kidney tubules of the "open" nephrons without passing through the glomerular filter; the "closed" nephrons can be used as controls.

Various proteins—casein, serum albumin and globulin, egg albumin—were injected into the peritoneal cavity of *Salamandra punctata* and *Necturus,* and were found to be taken up by the tubular epithelial cells of the "open" nephrons in the form of colloid droplets. None was present in the "closed" nephrons. The identity of the colloid droplets with the injected material was insured by coupling the various proteins with the disodium salt of 2 naphtol-3:6 disulfonic acid which has an intense red color.

Likewise the injection of fats and lipids was followed by fatty changes and the presence of cholesterol crystals in the renal epithelium of the "open" nephrons. The colloid droplets and fatty changes thus produced in renal epithelial cells were in no way different from those seen in nephrosis.

It is concluded that the colloid droplets and fatty changes seen in human nephrosis are due to reabsorption and storage of proteins and lipids passed through the glomerular filter into the tubules.

THE TITRATION OF TRACES OF ANTIBODY: A TECHNIC USING MAXIMAL SERUM PROPORTIONS WITH SECONDARY INDUCTION OF AGGREGATION. Herbert Lund (by invitation), Boston, Mass.

Abstract. A marked increase of sensitivity can be obtained by treating each of the two stages of serological aggregation as individual reactions. This is done by adjusting the proportions of serum and antigen to favor maximal sensitization (a maximum of serum and an arbitrary minimum of antigen) and secondarily inducing aggregation of the diluted antigen (by centrifugation and reconcentration). This differs from the usual methods which strive to accommodate the conditions of both stages simultaneously by using a single ("optimal") proportion of serum and antigen. The method was applied to the iso-agglutination reaction and (with the modification of lowering specific gravity by the addition of saline solution prior to centrifugation) to the flocculation test for syphilis. The sensitivity of these reactions was increased 32 to 64 times that of standard technics. The main quantitative relationships found were as follows:

1. Within a wide range, sensitivity is directly proportional to the amount of serum and inversely proportional to the amount of antigen used in the reacting system. There is no optimal zone.

2. Volume increase by the addition of saline diluent does not appreciably affect the combination of antigen and antibody. Only in the extremely large volumes is the efficiency of the reaction appreciably affected.

3. From the above direct proportion, a convenient method of expressing titrations is evolved. This is to state the calculated minimal volume of undiluted serum required to aggregate an arbitrary unit of antigen. This can be determined by using the above technic of maximal serum proportions and quantitatively titrating by progressive serum dilution, and can be calculated by the formula: volume of serum used times the dilution at the end-point divided by the arbitrary units of antigen used in the reaction.

By a preliminary clinical trial of the above method it was found that occult reagin could be detected and titrated in sera of latent and treated syphilitic patients and in the sera of many normal individuals.

THE FATE OF TUBERCLE BACILLI PHAGOCYTED IN VIVO AND IN VITRO BY MONONUCLEARS DERIVED FROM NORMAL AND IMMUNIZED RABBITS. Max B. Lurie, Philadelphia, Pa.

Abstract. A mixture of tubercle bacilli and India ink was injected intravenously into normal and tuberculous rabbits. Two days later, bone marrow containing both phagocytized bacilli and carbon particles was removed from each rabbit. Each specimen of bone marrow was divided into two portions one was cultured to determine the number of living bacilli present, the other portion was placed in the anterior chamber of the eye of a normal albino rabbit. The marrow derived from the normal rabbit was placed in one chamber; the cells from the immunized animal were placed in the other chamber of the same rabbit. Two weeks later the irides of both eyes with their growth of carbon-bearing mononuclears were removed and cultured. It was found that within the mononuclears derived from the normal animal the bacilli grew abundantly, while within those derived from the tuberculous animal the growth of bacilli was definitely inhibited, in spite of the fact that these cells were growing in a nonimmunized environment.

Mononuclears derived from sterile pleural exudates of normal and tuberculous animals were permitted to phagocytize tubercle bacilli and carbon particles *in vitro* in the presence of normal or immune serum. The fate of these bacilli was determined by again using the anterior chamber as an incubator for the cells that had ingested the bacilli. It was found that under these clearly defined conditions the mononuclears originating in an immune animal possess in themselves greater inhibitory properties on the growth of tubercle bacilli than cells derived from a normal animal. The addition of immune serum to normal cells or of normal serum to immune cells, under the conditions of this experiment, did not significantly change their inherent properties to influence the growth of bacilli within them.

CARCINOMA OF THE PARATHYROID GLAND. Karl A. Meyer (by invitation) and Alex B. Ragins, Chicago, Ill.

Abstract. A rare case of primary carcinoma of the parathyroid gland with disseminated fibrocystic disease of the bones is described. The extensive roentgenological and biochemical study is confirmed by autopsy. The influence of the carcinoma of the parathyroid on calcium and phosphorus metabolism is discussed.

MEAT EXTRACTIVES AND THE NONPROTEIN NITROGEN OF THE BLOOD. E. Mylon (by invitation) and M. C. Winternitz, New Haven, Conn.

Abstract. The ingestion of boiled ground beef, from which the fluid has been drained, causes a sharp elevation of the blood nonprotein nitrogen. This reaches the normal again only after 48 hours. When the partially evaporated fluid as well as the boiled beef are fed, the rise in blood nonprotein nitrogen is even less than after the same nitrogen content is fed in the form of raw ground meat. The meat extractive seems to contain essentials for the synthesis of the blood nitrogen.

SPECIFIC LESIONS OF THE SMALL INTESTINES IN CONGENITAL SYPHILIS. TWO ADDITIONAL CASES. Bjarne Pearson and Emil Palik (by invitation), New Orleans, La.

Abstract. Two cases of congenital syphilis involving the small intestine were observed among 1855 individuals of 1 year of age or less on whom necropsies were performed between January 1, 1937 and December 31, 1940. The lesions are bright yellow, annular plaques with occasional, superficial, central ulceration, and they are separated by varying intervals of normal intestine. Their average width is from 1 to 1.5 cm. Coalescence of the plaques to involve wider segments of the bowel can be seen. The intestinal wall beneath the plaque is thick and the serosa is covered with fibrin, causing loops of bowel to adhere to one another. A peritonitis may thus be present without perforation.

In the earlier lesions microscopic sections show mainly fibroblastic proliferation with foci of polymorphonuclear cells, lymphocytes and plasma cells in varying degrees. These "abscess-like, miliary" foci are constantly present in the intestinal lesions. In the more advanced lesions, section shows necrosis of the mucosa and predominant fibroblastic proliferation. Many *Treponema pallidum* were demonstrated in sections stained by the Levaditi and Steiner methods. As far as we know, with the three previously reported

cases by one of us (B. P.), these are the only cases recorded in the English literature which were proven by the demonstration of the specific micro-örganism.

THE METEOROGENESIS OF CEPHALIC MALFORMATIONS. William F. Petersen and (by invitation) A. Mayne, Chicago, Ill.

Abstract. Cephalic malformations may be caused either by recessive genetic or environmental factors effective during the earliest stages of differentiation. Presumably most such malformations are associated with a delay in the separation of the medullary plate and the notochord (Bonnevie). This may in turn be associated with the trend toward femaleness revealed by such malformation. In America malformations are more frequent in the northern tier of states and in adjacent states the annual trend-of-production curves reveals a high correlation coefficient.

An examination was made of the environmental conditions (temperature, barometric pressure, etc.) existing at the presumptive conception period of more than 1,000 cephalic malformations studied in the Chicago region. In general there appeared sufficient differences at various levels of temperature and barometric pressure in the frequency of malformation and of the total birth population at the same level or at least 1 day during the interval when conception presumably occurred to conclude that real differences did occur. Increases in malformations over the expected number occurred at just below normal temperatures in winter, spring and summer periods. In the autumn an excess of malformations occurred with great deviations from normal temperatures, whether positive or negative.

A RARE MALIGNANT TUMOR OF THE THYROID WITH POSTMORTEM FINDINGS. S. H. Polayes, Brooklyn, N. Y.

Abstract. G. C., a white male of about 80 years of age, had a thyroidectomy performed at the Cumberland Hospital of Brooklyn for the removal of a thyroid mass which had existed for a period of about 35 years and which, in the last 5 years, had enlarged rapidly. The mass was situated in the anterior portion of the neck and had caused difficulty in breathing, hoarseness and pronounced loss of weight, despite increased appetite. The heart was enlarged and fibrillated. The basal metabolic rate was +22. The blood examinations, including count, chemistry and serology, were all normal.

The resected tumor measured 23 by 10 by 7 cm. and was lobulated and partially encapsulated. It was firm and its tissue was yellow-gray and dense, with a tendency to the formation of small cystlike spaces and to calcium deposition. Pathologically the tumor was considered a spindle cell sarcoma originating in a fibro-adenoma of the thyroid. The patient made an uneventful recovery and was discharged from the hospital on the 18th postoperative day. The clinical diagnoses were as follows: tumor of thyroid (sarcoma or adenocarcinoma), arteriosclerotic heart disease, left indirect inguinal hernia, hypertrophy of the prostate with diverticulum of the bladder.

Five months later the tumor recurred to its original size and this mass had to be resected again. It measured 20 by 15 by 8 cm. and presented gross and microscopic features similar to those described in the previous mass. The patient died several hours after operation. The postmortem examination revealed, in addition to the sarcoma described above, acinar metas-

tases to the mediastinal and subpleural lymphatics as well as several interesting subsidiary findings. In addition the patient had hypertrophy of the prostate (adenomatous), dilatation of the urinary bladder (obstructive), multiple diverticula, adenomatous polyps of the gallbladder, cholelithiasis, adenomatous polyp of the rectum and fibroma (fibrosarcoma?) of the duodenum.

Representative sections of both thyroid masses were studied by the following pathologists: Drs. Douglas Symmers, Arthur Purdy Stout, Paul Klemperer, Allen Graham, Shields Warren, N. Chandler Foot, James Ewing, W. G. MacCallum and A. E. Hertzler. Almost all of the above pathologists concurred in the diagnosis of sarcoma. One called it a carcinoma; another called it a carcinosarcoma, and another did not commit himself.

MORPHOLOGIC APPEARANCE OF CEREBRAL ANOXIA. Gabriel Steiner, Detroit, Mich.

Abstract. Cerebral anoxia has four morphological characteristics: (1) pallor of the involved tissues, particularly of the cerebral intercellular substance, seen in hematoxylin and eosin and other stainings; (2) sharp demarcation of these anoxic areas against the adjacent tissues; (3) well-defined perivascular arrangement of anoxic areas or at least definite regional relationship to the vascular bed; and, negatively, (4) the absence of visible change of the vascular walls and the absence of inflammatory or glial reactions. The nerve cells may show an ischemic lesion, or they may show no detectable lesions. These findings suggest beginning necrobioses.

In the routine examination of 2,000 brains, I found 12 cases having anoxic spots as incidental findings. The 12 cases can be divided into three groups. The first group of 3 cases represents mechanical interruption of the blood supply either by ligation or by plugging of vascular lumina (1 case of ligature of the internal carotid after fracture of the mandible, 2 with capillary bacterial embolisms). The second group consists of 5 cases with marked generalized anemia (2 cases with lymphatic anemia, 1 with thrombocytopenic purpura, 1 with Banti's syndrome, 1 with carcinoma of the cervix with severe anemia). The third group consists of 4 cases of endogenous or exogenous toxic conditions (2 cases with uremia, 1 with eclampsia, 1 case in which death was due to general anesthesia). Not included in these groups are a number of cases of asphyxia found in a special investigation of the newborn, cases in which identical pictures of cerebral anoxia were seen.

The morphological criteria are well defined and specific; there are no lesions which are confusing. In the so-called spongy state of the cortex, which could be mistaken for an anoxic spot, the lesion is more diffuse; there is a coarse meshwork consisting of a dense network of glial fibers and large round or oval holes. The arrangement in the spongy state is laminar or pseudolaminar whereas the anoxic spots show a perivascular arrangement. One cannot expect to see anoxic spots until a certain length of time has elapsed. Twelve hours to several days after the causative damage are necessary. Particularly interesting are the pictures of bacterial embolisms. The first phase is indicated by bacterial masses plugging the capillary lumina and by strictly perivascular anoxic spots. The second phase is represented by leukocytic and bacterial diapedesis into these spots and the third phase by miliary or submiliary abscesses. Anoxic spots in cerebral bacterial em-

bolisms have to be considered a prerequisite in the pathogenesis of miliary abscesses. In 1 case (Banti's syndrome) a very late stage of anoxic spots could be seen in the nucleus caudatus. This late stage was represented by a perivascular glial scar with numerous fibril-producing astrocytes and by a complete loss of nerve cells. Occasionally anoxic spots may develop secondarily into definite softenings with mobile, compound granular fat cells. However, this late stage is seen only when the anoxic area surpasses its limited perivascular extension. Smaller anoxic spots are often seen with only the four characteristics mentioned and without any tendency for the mobile type of degeneration. Early changes may be considered reversible.

The problem of whether the morphological pictures described herein are due to a lack or deficiency of oxygen (so-called anoxia) or to a deficiency of other nutritional blood elements (dextrose, minerals, etc.) cannot be solved by morphological investigation alone. However, animal experiments and physiological data conform best with the conclusion that anoxemia with consequent tissue anoxia is the main factor in causing the characteristic morphological appearance in the central nervous system.

OBSERVATIONS ON THE DISTRIBUTION OF EXPERIMENTAL ATHEROMAS IN THE ARTERIES OF RABBITS. Sigmund L. Wilens, New York, N. Y.

Abstract. This communication describes an attempt to show that intimal lipid deposits found in areas of arteries, immobilized by being enclosed in silver cuffs, after chloresterol feeding has been instituted, are not due to the injury provoked by the cuff, but rather to the aggregation (or migration) of lipid from surrounding points in the intima to the immobilized zone.

METASTASES OF PRIMARY CARCINOMA OF THE BREAST. Otto Saphir, Chicago, Ill.

Abstract. The sites of metastases of 43 breast carcinomas are given. Special emphasis is placed on the occurrence of metastasis to the spleen, suprarenal glands and ovaries, which organs were involved 10, 19 and 7 times respectively. From the microscopic appearance of the primary tumor, no conclusion could be drawn as to the length of survival of the patients after radical mastectomy or the extent of metastases at the autopsy. However, the presence of isolated tumor cells regardless of the type of carcinoma, separated from basic structures of the carcinoma, indicates a high degree of malignancy. Emphasis is placed upon small and clinically unnoticed carcinomas of the breast which may give rise to widespread metastases. In this series there was no apparent difference in the survival period of patients with and without postoperative radiation treatment.

CARCINOMA OF CERUMINOUS GLAND. Shields Warren and Olive Gates, Boston, Mass.

Abstract. One case of adenocarcinoma of ceruminous glands is reported. It developed behind the right ear of a man, 78 years old. There was an ulcer 1.2 cm. in diameter. The tumor was 2 cm. in diameter and one part was cystic. On microscopic examination the cells showed a reticulated, foamy and finely granular cytoplasm. Fine droplets of fat were present. It somewhat resembled a carcinoma of the apocrine glands. One sure and 2 probable cases of adenocarcinoma and 2 adenomas of ceruminous glands have been reported previously.

THE ISOLATION OF "POLIOMYELITIC" STREPTOCOCCI FROM THE STOOL IN ACUTE EPIDEMIC POLIOMYELITIS. Edward C. Rosenow, Rochester, Minn.

Abstract. Serial dilutions were made at steps of 1:10,000 in rapid succession, alternately in tall tubes of dextrose-brain broth and soft dextrose-brain agar, of 10 per cent emulsions of stools. Streptococci always grew in low dilutions in mixture with *Bacillus coli* or other bacteria, irrespective of the source of the stools, but streptococci grew in high dilutions, usually in pure culture, most often when stools were obtained from patients during the acute stage of epidemic poliomyelitis, and less often during convalescence; only occasionally when stools were obtained from persons suffering from non-epidemic disease; and almost never when stools were obtained from well persons remote from cases of poliomyelitis. Streptococci isolated in high dilutions from stools of persons ill with poliomyelitis had characteristic virulence and specific agglutinating and precipitating properties. "Virus" takes were repeatedly obtained in mice with emulsions and filtrates of stools from patients ill with acute poliomyelitis if cultures from the stools yielded the "poliomyelitic" streptococcus.

Fourteen per cent of 229 mice inoculated intracerebrally with filtrates or intranasally with emulsions of stools from 46 cases of active poliomyelitis, 2 per cent of 55 mice inoculated with emulsions of the stools of 8 patients convalescing from poliomyelitis, and 1 per cent of 136 mice inoculated with emulsions of the stools of 41 well or ill persons remote from poliomyelitis died in 3 to 21 days from causes other than pneumonia. Death in "virus time" occurred in 21 per cent of 187 mice that received by serial passage brain emulsions, or filtrates of brain emulsions, of mice that died late after inoculation of filtrates or emulsions of stools from 14 cases of active poliomyelitis. Lesions in many of the mice that died late resembled those of encephalopoliomyelitis.

STUDIES OF NEUTROPHILIC MATURITY FOLLOWING INJECTION OF FRACTIONS OF STERILE EXUDATES (RABBIT). George H. Reifenstein (by invitation), Syracuse, N. Y.

Abstract. Sterile exudates were produced, following the methods of de Haan, Mudd and co-workers, and others, by the intraperitoneal injection of 0.9 per cent sodium chloride solution into rabbits. The supernatant fluid fractions of such exudates were injected intravenously into 11 other rabbits and leukocyte studies made. Previous studies (*Am. J. Path.*, 1941, **17**, 219) have shown that neutrophilic leukocytoses regularly follow several hours after such injection. In the present experiments, hourly determinations were made of the maturity of polymorphonuclear neutrophilic leukocytes in the circulating blood of these rabbits. Neutrophilic cells were classified in various groups of maturity according to the appearance of their nuclei.

Preliminary studies showed that the neutrophilic maturity of these and other rabbits studied without injection remained relatively constant during a 6 hour daily period. Following an intravenous injection of 5 cc. of supernatant fluid fraction of a sterile exudate, neutrophilic "left shifts" were constantly observed in a series of 14 experiments. Following repeated injections of 5 cc. of supernatant fluid fractions into the same rabbits, these "left shifts" were considerably greater and more progressive. Similar studies of

these same rabbits during the same daily period, following single or repeated control injections of 5 cc. of sterile sodium chloride solutions of equivalent concentrations showed no neutrophilic "left shifts." These "left shifts" were due to increases in the numbers of more immature neutrophils in the circulating blood, probably through release of these cells from the bone marrow. Although the nonfilamented neutrophils ranged from 70 to 90 per cent with maximum "left shifts," metamyelocytes very rarely were observed, and cells more immature than metamyelocytes never were found in the circulating blood.

These results seem to indicate that some substance or substances, not sodium chloride, present in supernatant fluid fractions of peritoneal exudates of sterile rabbits produced neutrophilic "left shifts" when injected intravenously into other rabbits. Following repeated injections of supernatant fluid there was an apparent "summation" of these "left shifts."

CENTRAL NERVOUS SYSTEM IRRITATION FOLLOWING INJECTION OF TESTICLE EXTRACTS. M. C. Winternitz and (by invitation) E. Mylon and R. Katzenstein, New Haven, Conn.

Abstract. Extracts of testicle, particularly, but those of kidney also, when injected into animals result in symptoms of irritation of the central nervous system. These may be associated with change in the coagulation time of the blood and can be suppressed by preliminary injection of small amounts of the same extract and by heparinization. Fractionation of testicle extract results in a nucleoproteid-lipid complex, the injection of which causes thrombi to be formed, but as the extract is purified the symptoms of irritation of the central nervous system become less.

By vote of the Council of the American Association of Pathologists and Bacteriologists this issue of *The American Journal of Pathology* honors H. GIDEON WELLS. The senior authors of the articles have all been pupils of Dr. Wells.